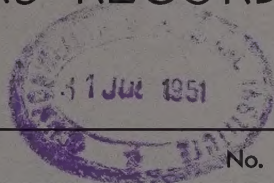


THE

# HAWAIIAN PLANTERS' RECORD



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Vol. LIV

1951

No. 1

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*Hawaiian Sugar Planters' Association*  
*For 1951*



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R. P. HUMBERT

# **Warfarin (Compound 42)**

***A Promising New Rodenticide for Cane Fields***

**By R. E. Doty**





# **Warfarin (Compound 42)**

## ***A Promising New Rodenticide for Cane Fields***

By R. E. Doty<sup>1</sup>

### **SUMMARY**

This paper presents our preliminary results obtained with warfarin (Compound 42) as a rodenticide in both field and cage tests.

These results have been so satisfactory that we are recommending it be given extensive trials on a plantation scale.

The success of this new material is entirely dependent on the cumulative effect of small doses taken over five to 10 days. In such low concentration, the rats do not detect the poison so no bait shyness has developed in either cage or field tests.

The use of this new poison eliminates the necessity of prebaiting with unpoisoned bait. The warfarin-treated rolled oats are applied to all field stations from the start and kept continuously available. Four or five days after the rats discover and begin eating the new food, they begin to die, and this killing process can go on as long as bait is present and new rats appear.

Experiments have shown that it may require as long as 16 days to obtain a 100 per cent kill of rats in the field, compared with nine to 10 days under our standard prebait treatment. This will necessitate additional field equipment but the actual labor requirement should be lessened.

In cage tests we found that rats ate an average of 36.3 per cent of their body weight of the warfarin (1-4000) rolled oat mixture and lived an average of 6.2 days after their first meal of the poison mixture.

The addition of 0.3 to 0.4 per cent of para-nitrophenol to the bait to prevent molding is recommended, thus making frequent inspection visits unnecessary.

Due to the low concentration of warfarin in bait, it is relatively safe from poisoning accidents, even when used around homes.

Warfarin bait is more expensive than any other raticide that we have used on a field scale. To balance this extra cost, we hope that we can credit a saving in labor and an increase in poisoning efficiency, mainly by the continuing poisoning process over the entire period of exposure instead of confining it to a short, definite poisoning period as under our prebaiting plan.

We do not believe that warfarin will replace all other rodenticides but it has a definite place in our rat control program. It will be especially useful along wasteland where there is almost continuous migration into big cane and in and around plantation homes where safety from accidental poisoning is a first consideration.

<sup>1</sup>R. E. Doty is associate agronomist, Experiment Station, HSPA.

## INTRODUCTION

Warfarin is an entirely new rodenticide (1) (2) (6) now being tested by the Experiment Station and cooperating plantations. The present paper is a progress report on information to date and is designed to give aid in determining how and when to use this new rodenticide. Results have been sufficiently outstanding that we are recommending it be given extended trials on a plantation scale by all interested plantations.

The active ingredient in this compound is W.A.R.F. Compound 42 or 3(alpha - phenyl - beta - acetyethyl) - 4 - hydroxycoumarin. It was discovered by Dr. Karl Paul Link of the University of Wisconsin. During the experimental trial stage this chemical was called Compound 42 but recently Dr. Link has renamed it warfarin. This chemical prevents blood from clotting and painless death comes from internal hemorrhage.

During the past two years, its rodenticidal possibilities have been tested extensively on the Mainland by the Fish and Wildlife Service and found to have wide use.

**ADVANTAGES** This material offers a new approach to the rodenticide problem in that a single feeding of warfarin is not fatal to rats or mice, but mortality is brought about through the cumulative effect of small doses taken over five to 10 days. Because of the low concentration used, there have been no cases of bait shyness by rats observed in either cage or field tests.

Thus, the poison may be applied in the standard feeding stations immediately with no pre-baiting necessary. And since warfarin is only very slightly soluble in water as well as tasteless and odorless, it seems to be ideal for our moisture-laden cane fields.

In this way, as soon as rats find the

food, they begin to be poisoned, and stations will keep killing rats as long as bait is present without the need for changing from unpoisoned to poisoned bait at a specific time.

However, the stations have to remain in the field 13 to 16 days instead of the standard nine to 10 days for the pre-bait and poison treatment. This will require extra equipment but should be more than offset by the possible saving in labor.

In addition, para-nitrophenol 0.3 to 0.4 per cent to prevent molding (3) may be added to the warfarin oats with no ill effect, thus making frequent inspection visits unnecessary.

Further advantage is that the use of warfarin in such low concentrations provides a safety factor for men and domestic animals because the chance of getting a lethal dose from a single accident is much less.

**TOXICITY** Experiments by Dr. Link have shown that chickens are notably resistant to warfarin at a level that would kill rats in 10 to 15 days. In isolated cases where a chicken appeared to be visibly affected, diet deficiencies were found to be chiefly responsible.

Dr. H. J. Spencer of the Fish and Wildlife Service, reporting at the Florida Pest Control Association Meeting (5, pp. 20-22), stated that chickens fed for 10 days on Compound 42 (warfarin) at a dilution of 1-4000, continued on their feet and laid eggs. But he noticed three eggs with blood hemorrhage spots in the yoke and that even two weeks after doses of Compound 42 had been stopped, some blood hemorrhage conditions were still found in the droppings, indicating some damage.

However, it is possible to kill any warm-blooded animal with warfarin mixtures provided that animal eats a large



enough quantity of bait over a period of a week or more. We found a dead mynah bird near one of our field stations.

If multiple doses of our standard bait containing 0.025 per cent (0.25 mg/gram or 1 to 4000) were eaten, 12 grams of this mixture daily for three or four days might provide a lethal dose to a 2.2-pound cat, or an 11-pound dog might be killed by a daily consumption of 60 grams for the same period. This potential hazard in Hawaii would be at a minimum because our rolled oats bait is not attractive to cats, dogs or mongooses.

**SECONDARY POISONING** The danger of secondary poisoning to other animals that may eat warfarin-poisoned rats is not considered serious. The Fish and Wildlife Service has reported that (4, p. 2) "a small number of dogs and cats have been killed after ingesting large quantities of bait or after they consumed unusually large numbers of dead rats and mice which were poisoned by the preparation."

Dr. Link showed a lack of fear on this score when he killed some of his chickens treated with warfarin and served them on his own dinner table. However, he tried this only once and did not eat war-

farin treated chickens over a long period of time which might make a difference.

We plan to study this question by feeding warfarin-poisoned rats to caged mongooses.

**VITAMIN K AS ANTIDOTE** Vitamin K has been reported to be a specific and highly effective antidote for (Compound 42) warfarin, provided treatment is begun promptly before serious symptoms have developed. However, this situation is not clear-cut as these statements were based originally on the use of Dicumarol, a closely related chemical. Dr. Spencer (5, p. 22) has pointed out "that he had not been able to save an animal which had been poisoned with an MLD<sup>2</sup> of Compound 42 and where administration of Vitamin K had been made after symptoms had appeared."

"In the case of accidental ingestion by humans," the Fish and Wildlife Service recommends that (1, p. 2) "vomiting should be induced at once and a physician called. Treatment by the physician should include transfusion with whole blood and intravenous and oral administration of Vitamin K preparations as in the case of hemorrhage caused by dicumarol."

## BAIT MIXTURE

**BAIT FORMULA** Bulk rolled oats were used exclusively in all tests conducted by this Experiment Station. For these experiments warfarin (Compound 42) was added to rolled oats at the rate of 1 to 4000 (0.025 per cent or 0.25 mg/gm.). This was accomplished by mixing 1 part "Dethmor"<sup>3</sup> and 19 parts of rolled oats. Finely powdered para-nitrophenol (0.3 to 0.4 per cent) was added to a portion of the mixes as a mold deterrent (4).

Mineral oil was used in the mix at the rate of one quart to 15 pounds of rolled oats to prevent the poisoned powder from sifting to the bottom of the container. (One quart to 20 pounds is satisfactory.)

The addition of about 2 per cent of raw sugar crystals (3, p. 125) will further increase the attractiveness of the bait, especially for the tree rat. However, no sugar was added to the bait used in the tests reported here.

<sup>2</sup>MLD=Minimum lethal dose.

<sup>3</sup>Dethmor contains 0.5 per cent warfarin. See "Supplies of Warfarin (Compound 42)" for discussion of trade names and contents (page 18).

**MIXING** In the tests, the easiest way to mix these ingredients and prevent dusting was by adding oil slowly to warfarin while stirring. This made a paste similar to cake batter which gradually thinned as more oil was added. Final mixing was completed with an egg beater. The oil and warfarin mixture was then added to the dry rolled oats in a rotary mixer, which was run for about 10 minutes or until the operator was certain of a uniform mixture.

**ALTERNATE MIXING PLAN** A good mix may be obtained by adding the dry powdered warfarin (Compound 42) and para-nitrophenol to the dry rolled oats provided all ingredients are placed in an absolutely airtight container. Then the container can be rotated or turned slowly end over end. After a thorough mixing while dry, mineral oil should be added and the whole mixed thoroughly again to absorb or hold down the dust.

## CAGE TESTS

We have completed cage studies on 66 wild rats using warfarin (1-4000) and have not had a single bait refusal. The method of procedure was the same as reported formerly (3, p. 145) in the study of other rodenticides.

In addition to water and sugar cane,

rats were fed unpoisoned rolled oats for a few days to develop the relation of daily consumption of bait to body weight (percentage of body weight). Then followed many exact measurements of the daily consumption of warfarin treated oats until the rats died.

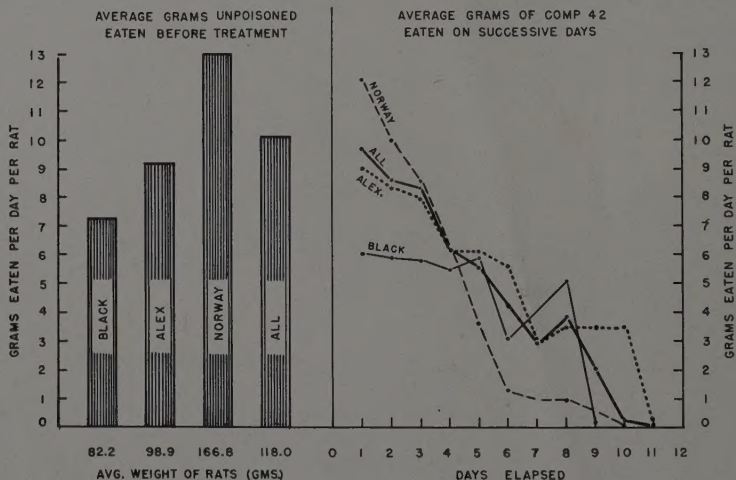


Figure 1. Consumption of warfarin by caged rats (from averages in Table 1). Record from 21 Norways, 35 Alexandrine, 10 Blacks, making a total of 66 rats.

**RESULTS** These measurements were tabulated and summarized in Table 1 and these data are illustrated in Figures 1 and 2.

The number of rats surviving each day

after feeding on warfarin is presented graphically in Figure 3. The distribution of rats dying each day after eating warfarin is shown in Table 2 and plotted in Figures 4 and 5.

**Table 1—Summary of Averages**  
**Showing the amount of unpoisoned and warfarin (Compound 42)**  
**poisoned oats eaten by caged rats.**

Species of rat		Norway	Black	Alex	Wtd. Avg. of all	% Rats Surviving
No. of each species studied		21	10	35	66	—
Avg. body wt. in grams		166.8±15.2*	82.2±11.3	98.9±5.2	118.0±5.8	—
Unpoisoned oats eaten	No. of days record	10.5	13.6	12.7	12.2	—
	Daily Avg. { In grams	13.2	7.3	9.2	10.2	—
	{ % of bdy. wt.	8.7±.5	8.9±.8	9.5±.3	9.2±.3	—
Compound 42 poisoned oats (1-4000) eaten in successive days	1st day	12.3	6.1	9.1		100
	2nd "	10.1	5.9	8.4		100
	3rd "	8.6	5.8	8.0		98
	4th "	6.2	5.5	6.2		97
	5th "	3.6	5.9	6.2		62
	6th "	1.7	3.1	5.6		47
	7th "	1.0	4.1	2.9		29
	8th "	1.0	5.2	3.5		18
	9th "	0.6	—	2.3		9
	10th "	—	—	3.5		5
	11th "	—	—	0.9		3
	12th "	—	—	0.0**		1.5**
Total poisoned oats eaten per rat (avg.)	In grams	38.6	32.0	42.3	39.6	—
	% of body wt.***	23.1±1.7	39.1±3.5	43.4±2.9	36.3±1.7	—

\* ± = standard error of mean.

\*\* One rat alive but not eating. Died on the twelfth day.

\*\*\* Statistics:

The difference in average amounts of poison eaten by Norway (*Rattus norvegicus*) compared to either Alexandrine—gray rat (*Rattus Rattus Alexandrinus*) or Black (*Rattus rattus rattus*), expressed in percentage of body weight, is highly significant.

For example, comparing Alexandrine with Norway, we have:

Alex 43.4±2.9  
 Norway 23.1±1.7

$$\text{Difference} = 20.3 \pm \sqrt{(2.9)^2 + (1.7)^2}$$

$$\text{or } 20.3 \pm \sqrt{11.30}$$

20.3±3.4 ∴ difference is 6 times greater than its standard error, giving high significance.

Likewise, the difference in the length of time until death between the Norway and the Alexandrine is also significant.

But the difference in average amounts of poisoned rolled oats eaten by the Black and the Alexandrine, expressed in percentage of body weight, failed to be significant. This is to be expected as the Black and the Alexandrine are almost identical in their habits.



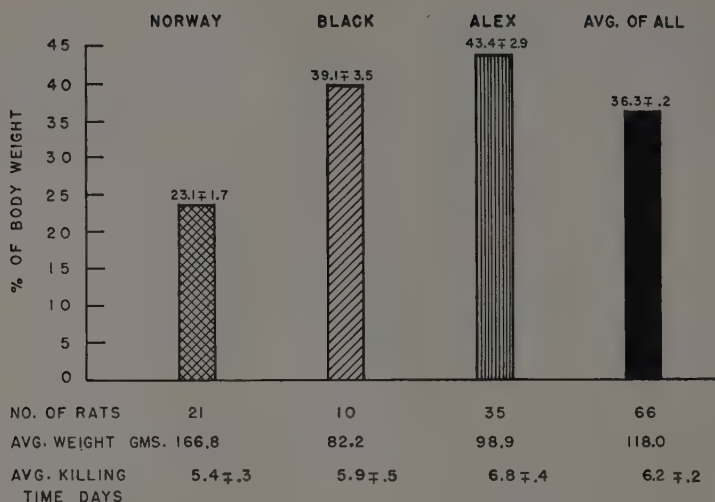


Figure 2. Total consumption of rolled oats treated with warfarin (Compound 42 1-4000) by rats before dying expressed as percentage of body weight (from Table 1 last line headed "% of body weight").

Table 2—Frequency Table  
Number and percentage of rats dying on successive days after their first meal of warfarin (Compound 42).

	Days elapsed											
	1	2	3	4	5	6	7	8	9	10	11	12
<b>ALEX—35</b>												
No. of rats each day	—	—	—	5	5	6	9	2	5	1	1	1
% each day				14.3	14.3	17.1	25.7	5.7	14.3	2.9	2.9	2.9
<b>BLACKS—10</b>												
No. of rats each day	—	—	—	2	2	3	—	3	—	—	—	—
% each day				20	20	30		30				
<b>NORWAY—21</b>												
No. of rats each day	—	1	1	3	5	5	5	—	1	—	—	—
% each day		4.8	4.8	14.3	23.8	23.8	23.8		4.8			
<b>TOTAL—66</b>												
% each day	—	1	1	10	12	14	14	5	6	1	1	1
Accumulated total		1.5	1.5	15.1	18.2	21.2	21.2	7.6	9.1	1.5	1.5	1.5
in %			1.5	3.0	18.1	36.3	57.5	78.7	86.3	95.4	96.9	99.9
<b>Mortality by Periods</b>	← 3% →			← 92.4% →						← 4.5% →		

Relative percentage of each species making up total per cent dying each day.

Alex.....	—	—	—	29.4	24.6	24.2	51.9	16.0	75.0	100	100	100
Black.....	—	—	—	41.1	34.4	42.3	—	84.4	—	—	—	—
Norway.....	—	100	100	29.4	41.0	33.5	48.1	—	25.0	—	—	—
Total.....	—	100	100	100.0	100.0	100.0	100.0	100.4	100.0	100	100	100

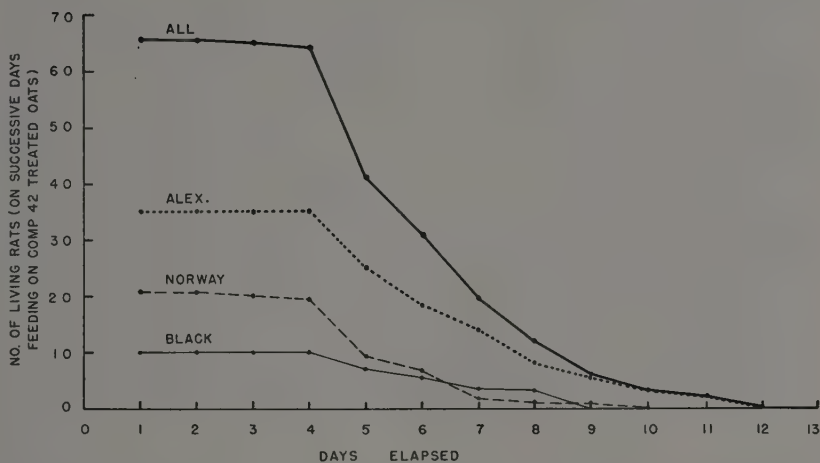


Figure 3. Number of surviving rats fed on warfarin (Compound 42) on successive days. Record from 66 rats of three species.

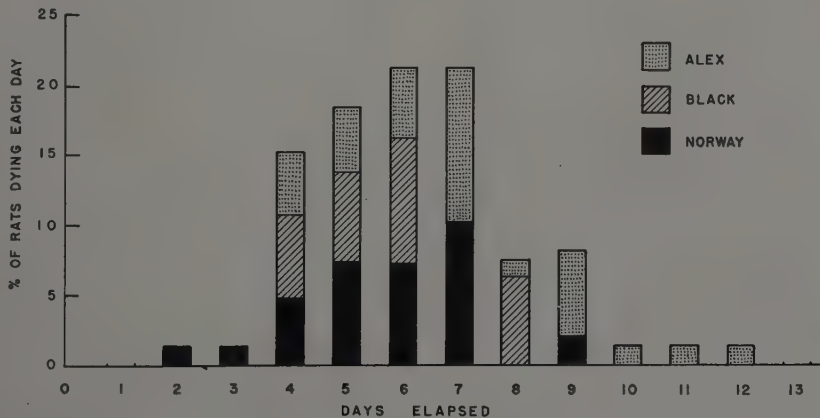


Figure 4. Percentage of rats dying each successive day following their first meal of warfarin-treated rolled oats. Record from 66 rats of three species. (From Table 2.)

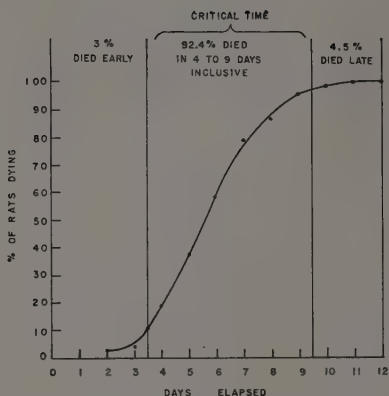


Figure 5. Accumulated totals of rat deaths by successive days expressed in percentages (from last line of Table 2).

**DISCUSSION** The total number of rats in this study is too small to draw final conclusions. The distribution table and curves have irregularities and blanks due to lack of sufficient numbers of specimens. But the urgency of securing some idea of the expected effectiveness of this poison under our field conditions has persuaded us to give what we have to date, as a progress report.

**CONSUMPTION OF BAIT** The unpoisoned oats eaten per day by the three species of rats in this cage study, computed in percentage of body weight (average 9.2 per cent from Table 1), agrees with previous measurements made in cages (3, p. 226). These studies gave consumption of unpoisoned oats ranging between 7.9 and 9.3 per cent of their body weight.

There was some variation in acceptance of the warfarin-poisoned oats. Many individuals ate as heartily of the warfarin mixture the first day or so as they did previously of the unpoisoned bait, while others ate less than they had of the unpoisoned. However, on the

average for the first two days of exposure (Table 1), the consumption of warfarin mixture was nearly equal to that of the unpoisoned rolled oats. Consumption declined rapidly as rats began to be seriously ill about 24 to 36 hours before dying. This condition is illustrated in Figures 1 and 6. The decline in the curve of consumption would be less pronounced for the first three days if it had not been necessary to use so many averages instead of individual daily consumption figures.

There were no rats in cages that refused the warfarin bait. This is the first poison we have tested that was not detected in some degree. Even thallium sulphate and zinc phosphide, the best field poisons we have, developed 3 to 4 per cent of bait-shy rats in former cage tests (3, p. 227, Fig. 73).

In many cases, the rolled oats in the feeding pans were bloodstained after the fourth day from hemorrhages developing while the rats were feeding. Postmortem examinations (see Appendix) indicated that hemorrhages developed in various parts of the body, that the blood became watery with black clots in it, and that internal organs were pale due to lack of red blood. The rats probably died from a lack of oxygen to their vital organs. (Figures 7-9).

**SUSCEPTIBILITY AND RESISTANCE** It is interesting to note that of the three species studied, the Norway appears to be most susceptible and the Alexandrine the most resistant to this poison. The Norway seems more susceptible to this poison because a lower percentage of its body weight of poisoned oats is required to produce death. An average of about 23 per cent of its body weight of poisoned oats produced death in the Norway compared to 39.1 per cent to kill the Black rattus and 43.4 per cent for the Alexandrine, resulting in an





Figure 6. Wild Black rat dying after being fed warfarin treated oats. Note the blood spot on the rolled oats in the bait can. Blood stains on the bait are quite common in field feeding stations.



Figure 7. This rat weighed 336 grams, ate 114 grams (34 per cent of its body weight) of warfarin treated rolled oats during a period of five days. It developed a severe hemorrhage from the left ear by the fifth day and died on the sixth day. Note: White rats were used as illustrations in order to show hemorrhage areas clearly.



Figure 8. This rat died after being fed warfarin (1-4000) treated rolled oats for six days. It developed a hemorrhage in the right rear foot and from the nose.

average of about 36.3 per cent for the 66 rats under study. Each of two Alexandrine rats actually ate 78 per cent of their body weights before dying. We conclude from our limited experience that the Alexandrine (gray rat) is the most resistant of the three species to this new poison. These figures are presented graphically in Figure 2.

**FIRST FEEDING UNTIL DEATH** In Figure 3 we have plotted the number of surviving rats feeding on the warfarin-treated rolled oats. This chart shows that only two rats (10 per cent) (both Norways) died during the first four days. Also only two Norways (10 per cent) survived for seven days. On the sixth day the survivors were: 18 (51 per cent) out of 35 Alexandrine, six (60 per cent) out of 10 Blacks, seven (33 per cent) out of 21 Norways, or a total of 31 (47 per cent) out of 66 rats (three species). Three Alexandrine (9 per cent) actually survived for nine days, the last

one of which did not die until the morning of the 12th day. The Norway averaged 5.4 days, the Black rattus 5.9 days and the Alexandrine 6.8 days until death after eating their first meal of warfarin rolled oats (Figure 2).

In Table 2 we have tabulated the number of rats that died each day following their first meal of warfarin. These figures have been converted to percentage of the total rats for easy comparison. The curve given in Figure 4 has been drawn from this distribution table representing a total of 66 rats.

This curve shows that the greatest number of rats died from the fourth to the ninth day after eating their first meal of warfarin. We have also calculated the percentage of rats dying each day after their first meal of warfarin, and given these in both Table 2 and Figure 5. We note that only 78.7 per cent of the rats in the cages were dead in seven days after their first feeding, and 95.4 per cent were dead in nine days. If we allow four days

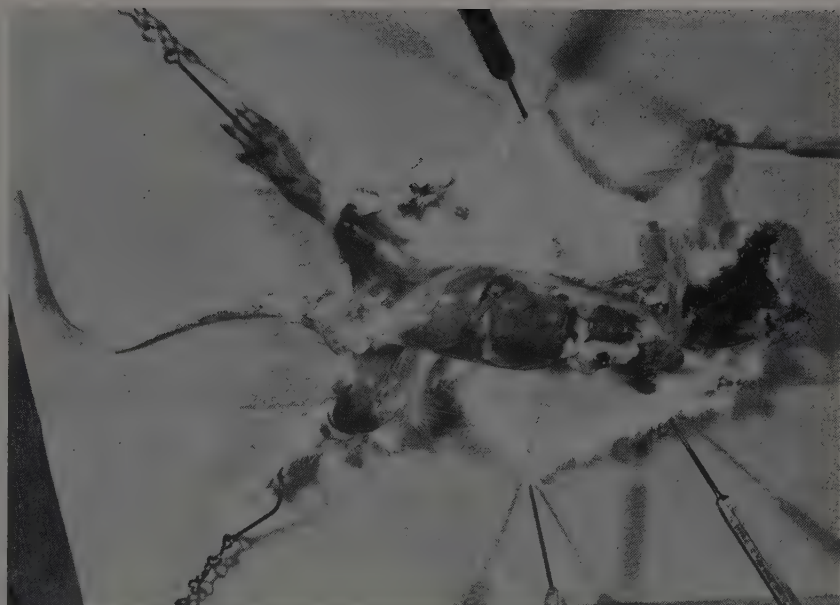


Figure 9. Same rat as in Figure 8. Autopsy showed severe internal hemorrhage in the musculature of the neck and both hind legs and the right hind foot. The liver was very pale indicating lack of a normal amount of blood.

for the average rat in the field to find a station and start eating, we must add four days to all of the above calculations for field application. To kill 80 per cent would require a total of 11 days, 95-96 per cent would require 13 days and a positive 100 per cent kill would require 16 days. This conclusion does not consider new migrations which, in the field, can happen at any time.

#### SUMMARY OF CAGE TESTS

1. Warfarin was never refused by any individually caged wild rat and was always fatal.
2. The amount of warfarin treated oats eaten before death calculated as percentage of body weight was 23.1 per cent for the Norway, 39.1 per cent for the Blacks, and 43.4 per cent for Alexandrine. These figures give an over-all average of

36.3 per cent of body weight, which may serve as a basis to calculate the weights of rats killed in the field when consumption is known.

3. The time required to kill after the first feeding of warfarin varied from a low of two days to a high of 11 days. The killing time averaged about 5.4 days for Norways, 5.9 days for Blacks and 6.8 days for Alexandrine, resulting in an average for 66 rats (three species) of about 6.2 days. We conclude that the Norway is most susceptible and the Alexandrine the most resistant of the three species.
4. Seven days feeding on warfarin treated rolled oats (1-4000) were required to kill 79 per cent of the rats, nine days to kill 95-96 per cent with an occasional rat (4 per cent) surviving to 10 or even 11 days.



5. A study of comparative cost (see Appendix) of materials for poisoning rats under our field conditions showed warfarin to be 50.7 per cent higher than thallium sulphate and 78.3 per cent higher than zinc phosphide. It is hoped that this extra

cost can be more than offset by savings in labor and by increased efficiency in the final killing power. It is also hoped that the basic price of the concentrated material will decline with increased production.

## FIELD EXPERIMENTS

Three extensive field tests were carried out on the island of Kauai. Experiments 62 and 63 were at Grove Farm Company Ltd. and Experiment 61 was at The Lihue Plantation Company Ltd.

The bait material for these tests was prepared as outlined under the heading "Bait Mixture."

In Experiment 62, 180 feeding stations were placed in a 61-acre field (Field No. 11C) of 14-month-old cane. Experiment 63 consisted of 120 feeding stations put

next to wasteland along the edge of Field 12 after harvest. Experiment 61 was in Field Hm 40B and consisted of 60 stations strung along an irregular field next to gulch wasteland.

To get an indication of the rat population in the area to be used for Experiment 62, unpoisoned rolled oats were put out for six days. Then warfarin treated oats were exposed for 13 days. Likewise, in Experiment 63, unpoisoned oats were exposed for six days before poisoned oats

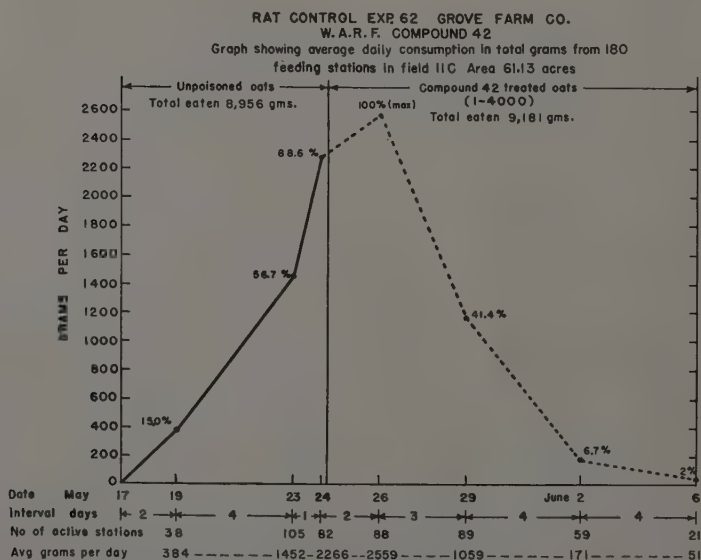


Figure 10. Note: The stations were put out on May 16 and 17, 1950, and removed on June 7, 1950.

GROVE FARM CO. FIELD 11C  
A6-EXP 62-RAT CONTROL-SHOWING NUMBERED FEEDING STATIONS  
WITH THE AMOUNTS OF COMPOUND 42 (DICUMAROL DERIVATIVE)  
BAIT CONSUMED AT EACH STATION

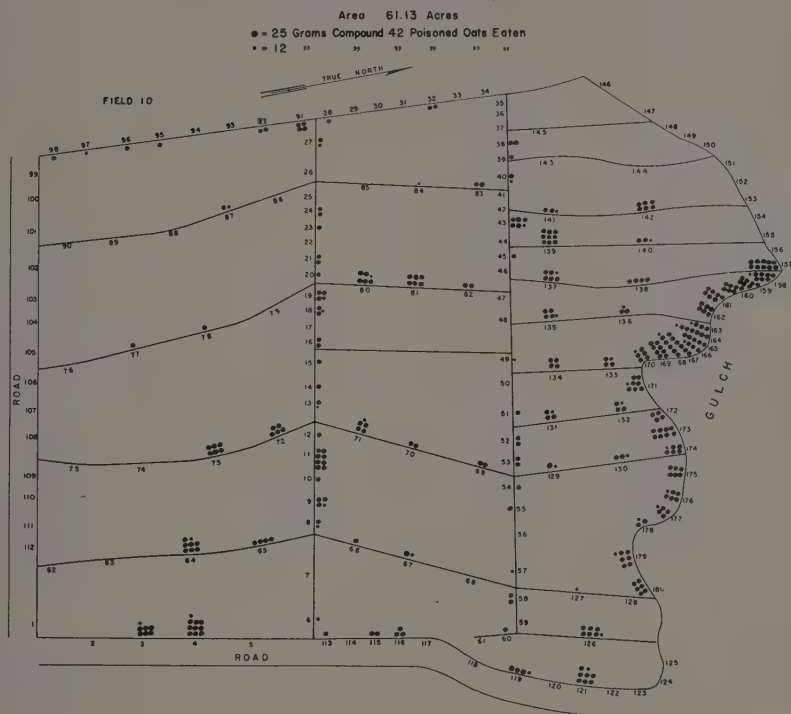


Figure 11.

were fed for 23 days. In Experiment 61, warfarin (Compound 42) treated oats were exposed for 35 days then unpoisoned oats were put out for 20 days, and poisoned oats again for 16 more days.

**RESULTS** The detailed data for Experiment 62 have been summarized and illustrated in Figures 10 and 11, and the data for Experiment 63 illustrated in Figure 12. Experiment 61 has been summarized in Table 3 and illustrated in Figure 13.

**DISCUSSION** Results show that rats ate the Compound 42 oats as well as they did the unpoisoned rolled oats. In Experiment 62, for example (Figure 10), the curve of consumption continued to rise after the change to Compound 42, and the average consumption of Compound 42 mixture during the first two nights of its exposure was greater than the consumption of unpoisoned oats during the last night of the unpoisoned period. However, beginning five days after Compound 42 mixture was put out

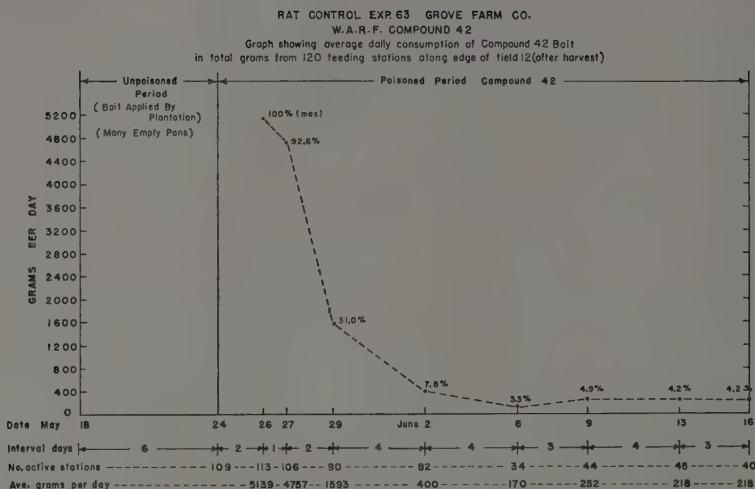


Figure 12. Experiment 63, Grove Farm Company Ltd. Field 12 E. Note: Stations were put out May 18, 1950, and removed June 16, 1950. The total consumption of unpoisoned bait during the unpoisoned bait period [May 18-24] represents token baiting in the majority of stations. Most stations were empty before Compound 42 bait was placed. The plantation record shows that more than 30.5 pounds (13,835 grams) of unpoisoned oats were consumed.

there was a sharp drop in the consumption, indicating that large numbers of rats were dead or dying. This decline continued for another eight days when consumption was almost nil. (Figure 10).

Attention is called to the distribution of the rat population (Figure 11) in this field. The greatest concentrations of rats are invariably directly related to migrations from gulches and wasteland where permanent cover is always present or from fields in process of being harvested where their cover is being destroyed.

The curve plotted (Figure 12) from the results of Experiment 63 declines in almost the same manner as in Experiment 62 during the first 13 days of the warfarin poisoned period. From this time until the end of the test (10 more days) the consumption never quite reached zero due to migrants coming from the

edge of the wasteland. Attention is called to the fact that in these two tests (Experiments 63 and 62), unpoisoned prebait was exposed in the stations for six and seven days, respectively, before warfarin (Compound 42) treated rolled oats was exposed. It is understood that in commercial practice, the warfarin treated oats would be applied directly at first. For an effective control then, it will be necessary to add about four days to the number of days mentioned above, i.e. 13 plus four or 16 to 17 days.

We now turn to study Experiment 61 in Field 40 B Hm of The Lihue Plantation Company Ltd. where warfarin treated oats were applied immediately to all stations as they were placed in a new area where nothing was known about the density of the rat population. We were very disappointed in this test at first, be-



RAT CONTROL EXPERIMENT 61  
LIHUE PLANTATION COMPANY Ltd., FIELD 40B  
W.A.R.F. COMPOUND 42

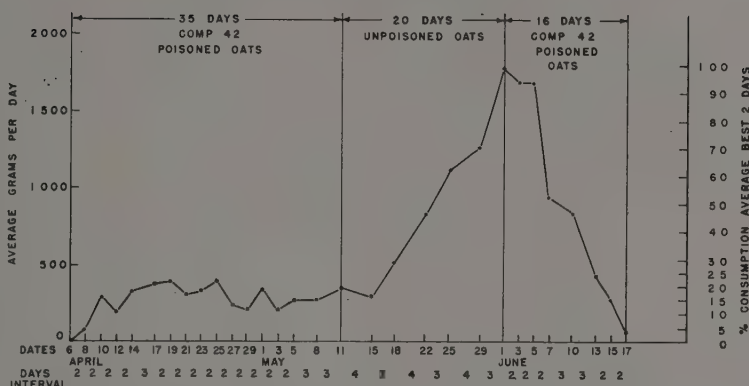


Figure 13. Average daily consumption of rolled oats in grams and percentage of highest day for 60 stations which had been treated as follows: First period (35 days) with Compound 42, Second period (20 days) no treatment, Third period (16 days) with Compound 42 again.

cause the rat population was so low that consumption was at a minimum, with many stations remaining inactive for many days. This fact could not be determined in advance.

However, by continuing this test for a much longer period than we originally intended, we learned a great deal. Compound 42 treated oats were exposed continually for 35 days. In this long period, we had numerous instances of stations remaining inactive during the first 10 days, suddenly becoming active for the next 10 to 15 days and then dropping to zero again. At a few stations some consumption developed a second time during the long period of exposure. *These facts indicated that rats were actively migrating from wasteland.*

A total of 23.3 pounds of Compound 42 treated rolled oats were eaten during this period (Table 3). On the basis of cage tests (Experiment 64—36.3 per cent of body weight), this amount of poison treated oats should have killed 64.3

pounds of rats or between 250 and 325 field rats.

Following this period of exposure of Compound 42, we placed unpoisoned oats in all stations for 20 days. Naturally, during this period of unpoisoned oats, all new rat migrants arriving at the stations became habitual feeders. In 20 days most stations became active and the consumption of unpoisoned oats increased to an appreciable total (Table 3 and Figure 13). This total of 1790 grams per day, average for the last three days of our unpoisoned bait, should represent between 200 and 250 average adult field rats eating regularly at our stations.

We decided to return again to Compound 42 to kill off all these new clients. During the first four days of exposure of Compound 42 treated oats, consumption remained very close to the peak but our record taken on the sixth day indicated a drastic reduction in average daily consumption. This rapid decline continued at a somewhat reduced rate the last 10

Table 3  
Experiment 61.—The Lihue Plantation Company Ltd.  
180 Stations started 4/6/50 and removed 6/17/50.

	Compound 42 Period 35 Days															Unpoisoned Period 20 Days					Compound 42 Period 16 Days								
	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
Interval (Days)	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2
No. of stations showing some acceptance	9	17	18	19	29	30	27	22	37	31	17	25	23	22	19	29	24	28	47	50	56	58	59	52	47	46	42	23	23
Tot. oats eaten (grams) active stations only	159	550	369	658	1072	743	607	680	800	465	403	703	408	561	828	1055	1202	1559	3328	3396	5012	5370	3378	3379	1882	2529	1310	567	153
Average daily consumption (gms.)	79	275	184	329	357	371	303	340	400	233	201	352	204	281	276	352	301	520	832	1132	1253	1790	1689	1690	941	843	437	284	77
% of highest daily consumption of unpoisoned oats in grams	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	.....	17	29	47	63	70	100	94	94	53	47	24	16	4
Grand Total = 10,058.6 gms. = 23.3 lbs.															G. Tot. Unpoisoned = 19,867.4 gms. = 43.8 lbs. Highest single day = 1789.8 gms.										Grand Total = 13,198.6 gms. = 29.1 lbs.				

days of the test. Consumption had declined to almost zero (5 per cent or less) at 23 stations and complete zero at 37 stations.

During this last period of exposure of Compound 42, an unforeseen feature developed. Just when the consumption was about nil at almost all stations and we were about to close the test, we observed that mynah birds were visiting a few stations and consuming small amounts of Compound 42 treated oats. The next day we found a dead mynah near one of the stations.

Undoubtedly these mynahs were the cause of some new consumption at a few stations just as we were closing the test. Since these stations were located along an open space between the cane and brush in fixed spots for such a long

period, it is not surprising that a few mynah birds got the "habit" too.

## **SUMMARY OF FIELD TESTS**

1. Warfarin treated oats were accepted in the field without noticeable detection or discrimination.
2. Warfarin treated rolled oats should be exposed in the field stations for at least 17 days or until consumption reaches zero. If there is active migration, small consumption of oats may continue.
3. Under a field poisoning program using warfarin, the feeding stations must be maintained in active operation much longer than with our standard practice of prebaiting and poisoning. This requires more station equipment, but should be more economical of labor.

## **GENERAL CONCLUSIONS**

1. We believe that warfarin will fit into our over-all field program. It is an additional tool which allows the plantation rat control supervisor greater latitude in selecting the most suitable poison for a particular set of conditions. We do not feel that the advent of warfarin automatically makes all other control methods obsolete. Many cases will occur where the use of other rodenticides is still desirable to produce more immediate kills and to reduce heavy infestations.

2. Warfarin is ideal as a follow-up treatment to clean up persistent infestations being constantly renewed by migrations from waste areas. We believe that a plantation system using lines of fairly permanent stations along gulches or wasteland where migration is more or less continuous, would be very effective. The use of para-nitrophenol (3) in the oats to keep them from molding will permit long intervals between visits

made necessary only to replenish the bait supply. As new rats migrate to the edge of the cane field, they would find the stations, become habitual eaters and die in about a week. This process could go on continuously while the cane matures. This plan should be especially useful for seedling variety plantings where rats accumulate and do damage during the periods between our standard prebaiting rounds.

In areas where migration is less of a problem, the use of paper feeding stations as developed by Waialua Agricultural Company Ltd. and the Honolulu Paper Company, which can be filled with bait and not recovered may be a real economy in labor (Figure 14).

3. Warfarin is especially desirable for home use in towns and villages on account of its additional safety factor which is not available in other effective rat poisons.

## SUPPLIES OF WARFARIN (COMPOUND 42)

Warfarin (Compound 42) is manufactured under license only by S. B. Penick and Company, 50 Church Street, New York 7, N. Y., under the trade name "Dethmor" and by R. J. Prentiss and Company, 110 William Street, New York 7, N. Y., as "Rax Powder (Compound 42)."

These prepared mixtures contain 0.5 per cent of Compound 42 in a carrier of finely ground corn meal or starch. This raticide has now been released for general use and may be purchased without restriction. The price has been quoted at \$1.95 per pound f.o.b. New York.

The Ralph H. Marlowe Company is the territorial distributor of "Dethmor" for the S. B. Penick and Company. The distributor for R. J. Prentiss and Company is the Honolulu Chemical Company Ltd. Both companies are located in Honolulu.

Arrangements are now being completed to furnish this raticide in 5 per cent as well as 0.5 per cent to processors and large users of this material. This will be a distinct aid for the plantation mixing plants as they can use the same methods formerly used in mixing thallium sulphate or zinc phosphide. Using 0.5 per cent warfarin at a rate of 1 in 20 gave too much starch in the oil mixture. To alleviate the pasty characteristics of the starch-oil mix, there was a tendency to increase the amount of the oil carrier.

The Pacific Chemical and Fertilizer Company is marketing warfarin in an oiled rolled oats-para-nitrophenol mix ready to use in 150-pound drums at 36 cents<sup>4</sup> per pound and in less than drum quantities at 46 cents per pound.

A study of comparative costs of poisoned oats made up with zinc phosphide, thallium sulphate and warfarin is given in the appendix to this paper.

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## ACKNOWLEDGMENT

We are indebted to the Agricultural Departments of The Lihue Plantation Company Ltd. and Grove Farm Company Ltd. and the Island Representative Office, HSPA, for their whole-hearted cooperation in measuring and recording the detailed data on the many routine visits to the feeding stations.

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<sup>4</sup> Pacific Chemical & Fertilizer Company Circular Letter No. 7, dated September 29, 1950.



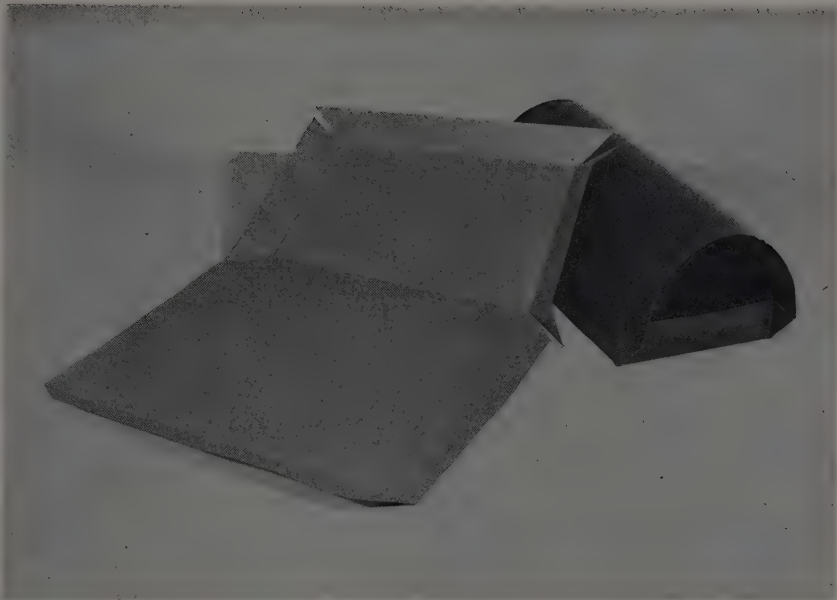


Figure 14. Waxed chipboard paper feeding stations developed by the Waialua Agricultural Company Limited and the Honolulu Paper Company.

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- <sup>5</sup>Gresham, William B. Jr. 42, 497, 1080 Figure in Recent Successful Florida P.C.A. Meet. Pest Control. 18: 20-22, 24. February 1950.
- <sup>6</sup>Krieger, C. H. Compound 42—a New Anticoagulant as a Rodenticide. Pests and Their Control. 17: 24, 26, 28. May 1949.

# Appendix

## COMPARATIVE COSTS OF MATERIALS FOR POISONING RATS AT HONOLULU, HAWAII

(Based on Pacific Chemical & Fertilizer Company's prices of November 8, 1950.)

Assuming costs of materials as follows:

Unpoisoned rolled oats.....	13.2¢ per pound
Zinc phosphide treated oats.....	24.0¢ " "
Thallium sulphate treated oats.....	46.0¢ " "
Warfarin treated oats, also contains para-nitrophenol	36.0¢ " "

Also assuming that rats eat 8.5 per cent<sup>(a)</sup> of their body weight of unpoisoned rolled oats per day (no spoilage or handling losses are included), to kill 100 pounds of rats, bait costs would be as follows:

### 1. For Zinc Phosphide:

Six days prebait at 8.5 per cent per day = 51% of body weight.	
$51\% \times 100 \times 13.2¢$ per pound =	\$ 6.73
(Rats eat 2.5% body wt. of zinc phosphide treated oats)	
Poisoned bait $.025^{(b)} \times 100 \times 24¢ =$	.60
	<hr/>
	\$ 7.33

### 2. For Thallium Sulphate:

Six days prebait at 8.5 per cent per day = 51% of body weight.	
$51\% \times 100 \times 13.2¢$ per pound =	\$ 6.73
(Rats eat 4.2% body wt. of thallium sulphate treated oats)	
Poisoned bait $.042^{(b)} \times 100 \times 46¢ =$	1.93
	<hr/>
	\$ 8.66

### 3. For Warfarin (Compound 42):

36.3 per cent of body weight required to kill.	
$.363 \times 100 \times 36¢$ per pound =	\$13.07
(This cost is 50.7 per cent higher than thallium sulphate and 78.3 per cent higher than zinc phosphide.)	

(a) Doty, R. E. Rat Control on Hawaiian Sugar Cane Plantations. Hawaii. Planters' Rec. 49: 225. Table XLV, and 226, Table XLVI. Also in Bul. 55 Agricultural and Chemical Series. Hawaiian Sugar Planters' Association Experiment Station. 1945.

(b) Ibid. p. 176, Table XXIV and p. 226, Table XLVI.

## NOTES AND COMMENTS ON RATS DYING FROM WARFARIN (COMPOUND 42) POISONING

### Experiment No. 64 — Cage Tests

Dr. A. R. Lamb, associate biochemist, cooperated with the writer in chloroforming a few of the very sick rats and making post-mortem notes as follows:

Rat #3 (Alex)—Days on Warfarin (Compound 42) until dead: 7 days

This rat developed a conspicuous eye hemorrhage about two days before death. The whites of the eyes became fiery red and then black. The whole side of head and neck filled up with thin watery blood. Liver pale, mottled; lungs normal. All abdominal viscera anemic, intestines empty. All tissues engorged with extra vascular blood. All blood was thin and watery.

Rat #4 (Alex)—(6 days)

This rat dragged his hind legs. Its movements became sluggish. The eyes were milky instead of dark and clear.

We dissected the rat and examined the body. There was much hemorrhage in the flanks and inguinal region, extending down the thighs. The liver and lungs were very pale. The blood from the tissues was too liquid and would not clot.

Rat #6 (Alex)—(6 $\frac{3}{4}$  days)

Examination of this rat showed two small subcutaneous hemorrhages along the flank. Liver slightly mottled; stomach packed full of intestinal round worms. Lungs slightly pale; heart appeared normal. Not much blood in evidence, all of which was watery.

Rat #9 (Alex)—(7 $\frac{1}{4}$  days)

Examination of carcass showed hemorrhage along the back just under the skin. Internal organs appeared normal. Diaphragm showed slight hemorrhage.

Rat #13 (Alex)—(5 days)

This rat paralyzed in rear quarters so hind feet dragged as the rat attempted to walk. Rat died that night.

Rat #15 (Alex)—(9 days)

Right rear foot swollen; full of subcutaneous blood (hematoma). Head and neck much swollen, full of serous unclotted blood. Liver pale pink. Chronic lesions on lung.

Rat #17 (Alex)—(6 days)

This rat had subcutaneous hemorrhage all along left side of the back; full of serous unclotted blood. The liver was pale and mottled. Lungs almost white; heart pale as were the other organs.





# Controlling Pineapple Disease Of Sugar Cane<sup>1</sup>

By C. A. Wismer<sup>2</sup>

## ABSTRACT

The pineapple disease organism, *Ceratostomella paradoxa* (de Seynes) Dade, is the principal cause of cane seedpiece rotting in Hawaii.

When the seedpieces are inoculated with the disease organism, best control is given by pyridylmercuric acetate, pyridylmercuric chloride and phenyl mercuric acetate (PMA). Ceresan is less effective. PMA is not only as effective as any other fungicide, but it is also less expensive on a field scale.

Field tests are of no value unless the disease organism is present. Because of the uncertainty of whether or not soil is infected with the organism, plantation tests to evaluate the different fungicides are apt to be valueless. However, they should prove extremely helpful in determining if the disease is present and therefore if treatment is needed.

Excellent protection of cuttings from rotting has been obtained in the laboratory when PMA (10 per cent aqueous solution) is added to the hot-water treatment (HPMA) at the rate of  $\frac{1}{2}$  pint per 100 gallons water.

Treating with HWT alone or with the fungicide after the hot-water treatment (HWT+PMA) gives less protection than does the fungicide alone.

Varieties respond differently to the hot-water treatment (HWT, 50° C. for 30 minutes or 52° C. for 20 minutes). Germination for 37-1933 after using HWT was improved while there was no response for 38-2915.

Recommended applications of PMA are one quart per 100 gallons of water for dipping or spraying cuttings and  $\frac{1}{2}$  pint per 100 gallons of water for HPMA.

The PMA treatment should by all means be used when cuttings are planted under unfavorable germination conditions—especially during dry or very wet soil conditions and cool temperatures.

<sup>1</sup> A thesis submitted to the graduate faculty of the University of Minnesota in partial fulfillment for the degree of doctor of philosophy, October 1950.

<sup>2</sup> C. A. Wismer is associate pathologist, Experiment Station, HSPA.

## INTRODUCTION

The pineapple disease organism, *Ceratostomella paradoxa* (de Seynes) Dade, is the principal cause of rotting of sugar cane cuttings (seedpiece) in Hawaii as well as in many other cane sugar producing countries. It occurs in almost every sugar cane growing area of the world (14,16,29,39,57). The disease has been reported as causing severe damage to sugar cane cuttings in Java, the Philippines, Puerto Rico, Hawaii, South Africa, the West Indies, Australia and has caused some damage in Taiwan (2,10,16,32,41,46,50,51,52).

*Ceratostomella paradoxa* causes the most damage to cuttings when planting is done during seasons which are less favorable for germination<sup>3</sup> of the buds, as in the fall and winter months. During recent years expanded programs by many Hawaiian plantations have necessitated planting during periods unfavorable for germination with subsequent losses due to poor germination. Since it is costly to replant a field, it is important to adopt measures which will produce a maximum number of shoots from cuttings planted by protecting them from rotting by *C. paradoxa*.

Treatment of cuttings with Ceresan<sup>4</sup> has been practiced by plantations in Hawaii for many years. However, treatment with this fungicide has not always given satisfactory results. The development of many new fungicides in recent years has added considerable impetus to the work of finding a fungicide which would more effectively and more economically reduce the losses from pineapple disease to sugar cane cuttings in Hawaii. This paper gives results of laboratory and field studies of some of the newer fungicides for the control of rotting of sugar cane cuttings by *C. paradoxa*.

## HISTORICAL SUMMARY

**THE PINEAPPLE DISEASE ORGANISM** The pineapple disease organism was originally studied by de Seynes (19,20) in France in 1886 where the organism was observed to cause a rot of pineapple fruits. He described and named it *Sporochisma paradoxum*.

Saccardo (55) in 1892 gave a short description of the organism which he listed as *Chalara paradoxa* (de Seynes) Sacc.

Went (71,72,73) in 1893 recognized the fungus on sugar cane in Java, described it and named it *Thielaviopsis ethacetica* Went. He used the name "pineapple disease" because the odor given off by sugar cane sections in the early stages of rotting by this fungus reminded him of the odor of the pineapple fruit. After a series of tests, Went

concluded that the odor was due to ethyl acetate formed by the action of the fungus.

Massee (39), working at Kew in 1893 with diseased sugar cane material from the West Indies, reported that *Thielaviopsis* sp. and *Melanconium* sp. which he isolated were only stages in the life cycle of *Trichosphaeria sacchari* Mass. In 1907 Lewton-Brain (33) reported obtaining spores of *Thielaviopsis* after sowing spores of *Melanconium* in culture medium. In later tests, however, he was unable to repeat these results and they were not substantiated by other workers (17,29,53,73).

*Sporochisma paradoxum* was found on the coconut palm in 1904 by von Höhnelt (70). He believed it identical with *Thielaviopsis ethacetica* Went. This

<sup>3</sup> The term "germination" is used in this paper to signify the development and growth of the buds on sugar cane cuttings or portions of the stalk.

<sup>4</sup> Ceresan, whenever mentioned in this paper, is understood to refer to Ceresan 2% ethyl mercury chloride.

was confirmed by Went from a specimen submitted to him by von Höhnelt and the fungus was renamed *Thielaviopsis paradoxa* (de Seynes) v. Höhn.

It was not until the work of Dade in 1928 (17) that the perfect stage was described and the fungus renamed *Ceratostomella paradoxa* (de Seynes) Dade.

In 1909 Cobb (15) found pineapple disease on all Hawaiian plantations.

Besides its effect on sugar cane it has been reported as being parasitic on pineapple, banana, coconut palm, and Palmyra palm (9,16,19,20,52,53,70).

## TREATMENT OF CUTTINGS WITH FUNGICIDES

1. *Early recommendations.* Went (73) in 1896 reported that the means of preventing pineapple disease in cuttings was to protect the cut surfaces with tar. Where this was done the "disease has ceased to show itself." Howard (29) in 1903 suggested dipping cuttings in Bordeaux mixture and then tarring the ends.

Cobb (13) in 1905 stated that in Queensland, Australia, cuttings were sometimes dipped in weak carbolic acid, while in the West Indies the cuttings are tarred at the ends.

Petch (53) in 1910 tested the effects of 19 chemicals on the spores of *C. paradoxa*. He listed the following in the order of their fungicidal value: mercuric chloride, formalin, carbolic acid, and copper sulphate. Copper sulphate was reported as having a "remarkably low" fungicidal value for *Thielaviopsis*.

2. *In Hawaii.* In 1905 Cobb (13) suggested the use of Bordeaux mixture for treating cuttings but did not specify any organism against which it was to protect the cuttings. Lewton-Brain and Lyon (34) in 1908 reported Bordeaux mixture to be the most satisfactory for protecting cuttings from rotting by the pineapple disease organism.

Moir (48) in 1922 noted injury to the

buds after treating cuttings with bichloride of mercury, 1:1000, Bordeaux mixture 5-5-50 and a copperstarch paste. Flowers of sulfur dusted on the ends had no effect on "germination" of buds.

In 1931, Urata (65), in small-scale experiments, found that treatment with sulfur and with Ceresan increased the percentage of "germination" of seed-piece eyes (buds).

Sudduth (58) in 1932 found that treatments with Semesan 1:50 solution at 52° C. for 20 minutes were more beneficial than the "dip" treatment. He also noted a stimulation of buds when cuttings were treated with bichloride of mercury 1:1000 and formalin 1:50 at 52° C. for 20 minutes. Bordeaux gave good results but copper fungicides as a rule proved quite toxic to the cane shoots. No rotting was found where boiling tar was applied to the ends of the cuttings.

Urata (66), in 1932, obtained a response with Ceresan treatment and later stated that a 1 per cent Ceresan solution in water was one of the cheapest and best materials for treating cuttings. Semesan gave similar results to Ceresan but was higher in cost. He reported that coal tar was not consistent in its performance, giving good results with some varieties and exceedingly poor results with others. He also reported poor results with bitumuls, creosote and carbolic acid. In another test sodium arsenite solution was found to be toxic to cuttings (67).

In 1933 Urata (68) recorded results of treating cuttings with eight mercury fungicides. The better ones were phenyl mercury acetate, ethyl mercury chloride (Ceresan) and ethyl mercury phosphate. In a second test, these three fungicides performed about equally well (69).

After further testing, treatment of cuttings with Ceresan was adopted as standard practice at the Kailua Substation of the Experiment Station, HSPA, in 1936, and in 1939 the Genetics

Department of this Station issued a memorandum to all Hawaiian sugar cane plantations giving their recommendations for the use of Ceresan for treatment of cuttings (25).

In 1950 the Pathology Department of the Experiment Station, HSPA, recommended phenyl mercuric acetate instead of Ceresan for treating cuttings on Hawaiian plantations. This recommendation was based on the results of the studies reported in this paper.

Phenyl mercuric acetate has also been used as a fabric preservative, a germicide in the paper industry, in paints, in tanning, for the prevention of sap stain, the control of crabgrass and has been found effective against apple scab, bitter and black rot of apples and brown rot of cherries (5,18,61,64).

3. *In Other Areas.* Bordeaux mixture was generally recommended for treating sugar cane cuttings prior to 1931 (1,13, 29,32,33,52,56). Since that time many new fungicides have been tested in the hope of finding one with greater efficiency and ease of preparation and application. Organic-mercurial fungicides have been found to be the most effective in the control of rotting caused by *Ceratostomella paradoxa* in Australia, Taiwan, Mauritius and South Africa (11,21,22,23,30,31, 42,43,45,46,47,54,72).

Since 1936, McMartin in South Africa has made a series of fungicidal tests with sugar cane cuttings (41,42,43,44,45,46, 47). He states that of the many disinfectants tested the ones containing some form of organically combined mercury have given the most satisfactory results. In 1946 McMartin (45) found that cuttings treated with a suitable fungicide germinated as well in dry soil as non-treated cuttings planted in soil with optimum soil moisture. Cuttings treated with Aretan and planted in dry soil conditions gave better results than any of

the other fungicides tested (44).

Weihe (74) in Mauritius in 1940 studied the effects of Ceresan and Bordeaux mixture on germination of sugar cane cuttings in localities where pineapple disease was common. Treatment with both stimulated germination. Evans (21,22) and Rochescouste (54) showed that organo-mercurial treatments increased the percentage of germination. Aretan gave the best protection of cuttings for the fungicides tested under both moist and dry conditions (21).

In Australia, Bell (3) in 1942 reported good germination following the use of mercurials. Mungomery (49) in 1947 stated that mercurial preparations hastened the rate of germination and protected the cuttings against *Thielaviopsis paradoxa*. Hughes (30) in 1948 and Hughes and Christie (31) in 1949 reported that the mercurials in general protected cuttings against soil organisms better than the other compounds tested.

Chu and Wang (11) in Taiwan in 1949 reported that treatment with 0.1 per cent Ceresan for two minutes and 0.1 per cent Granosan for 10 minutes were as effective as bichloride of mercury in killing *C. paradoxa*. They reported that the basic copper sulphate powder was ineffective.

**STIMULATION OF SUGAR CANE BUDS** The hot-water treatment stimulates germination of buds of sugar cane cuttings better than other treatments tried.

The value of the hot-water treatment in increasing percentage of germination and in shortening the time required for emergence from the soil was first demonstrated by Brandes and Klaphaak in 1920 (8). The cuttings were immersed in water at 50° C. for 30 minutes. In addition to the stimulation of germination, they reported the treatment also con-



trolled all forms of insects. Experiments dealing with the hot-water treatment were started independently in Java and Cuba in 1921 (8).

Field trials by Taggart with the hot-water treatment confirmed the work by Brandes. (75).

In 1930, Martin found that the hot-water treatment, 52° C. for 20 minutes, controlled chlorotic streak as well as hastened germination and stimulated growth of shoots (37).

Bell and Leece (4) in Australia reported that "warm water treatment of cane (20 minutes at 125° F.) considerably accelerates germination."

Varietal differences in response to the hot-water treatment have been reported (6,62). Wilbrink, in Java, concluded that the cane variety determines the temperature which can be used without killing the stalk itself. "A hot-water bath below 50° C. is always a stimulant to germination." (6).

Comments by Martin in 1932 concerning the hot-water treatment were as follows: "From this observation test it seems apparent that the hot-water treatment is detrimental to the germination of certain varieties. . . .Where the plants were extremely chlorotic many of the cuttings were removed from the soil and examined. Almost in every case the cuttings were found to be badly discolored and soured by the entrance of organisms. It was strongly indicated

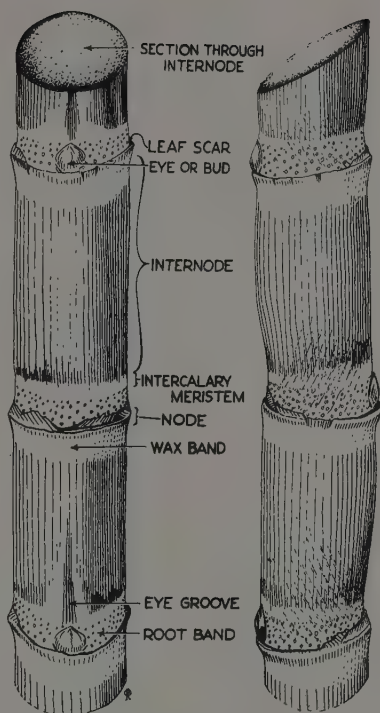


Figure 1. Cane cuttings or portions of sugar cane stalk used in planting. After Martin (36).

from these observations that the treatment (hot-water treatment) brought about conditions favorable for entrance and development of certain organisms which resulted in a rotting. . . .of the cutting." (62).

## PROPAGATION OF SUGAR CANE PLANTS

Sugar cane, *Saccharum officinarum*, Linn., is propagated commercially by planting sections of the stalks called cuttings, also known as seedpieces, setts, or points. During the development of the stalks, lateral buds are produced alternately at the nodes together with a root

band including root primordia (Figure 1). When cuttings, usually with two or more buds, are placed under favorable conditions, the buds germinate to produce new plants; root primordia develop roots which function until shoot roots are produced (Figure 2).

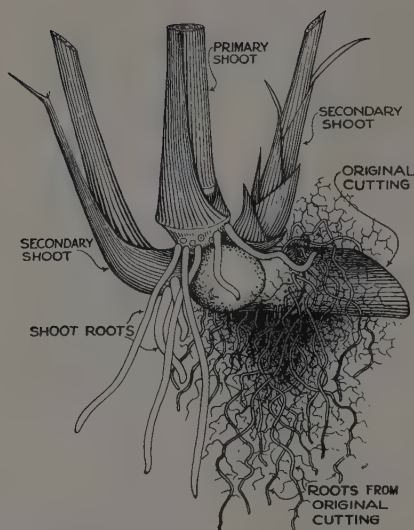


Figure 2. When a cane cutting is planted new shoots develop from the lateral buds and roots develop from the root band (See Figure 1). Later, stalks of the various orders and shoot roots develop. After Martin (36).

The cane rind effectively protects the interior of the stalk from microorganisms. When the stalk is cut into sections for planting, however, the ends are not protected. When conditions favor rapid germination of the buds there is some evidence that antibiotic substances produced by the cuttings delay penetration of rotting organisms. However, if germination is delayed because of unfavorable conditions such as age of buds, depth of planting (4,7), cold (4,12,16,41), wet conditions (4,16,30), or insufficient moisture for germination (1,22,30,41,44,45), the antibiotic substances are not produced and the rotting organisms can penetrate the ends of the cuttings, ramify the tissues and cause death of the buds or weakening of the shoot. Therefore, any factor that delays the invasion of the cuttings by microorganisms and stimulates the germination of the buds is important in the economic production of sugar cane.

## MATERIALS AND METHODS

**FUNGICIDES TESTED** More than 60 fungicides were tested in the laboratory to obtain preliminary indications of their possible value for field trials. The fungicides tested were furnished by chemical companies listed in Table 1 of the appendix.

**PRELIMINARY TESTING OF ISOLATES** About 70 isolations were made from cuttings with varying degrees of rotting in the soil. Bacterial and fungus isolates were initially tested

for pathogenicity by sterilizing a short section of stalk or cutting by flaming and puncturing it with a sterile needle, then introducing the isolate into the sterile puncture. The inoculated cuttings were then kept in a moist chamber at room temperature for five days, after which they were split and the amount of rotting recorded.

Isolates which caused some rotting in the preliminary tests, comprising two bacteria and three fungi, were further

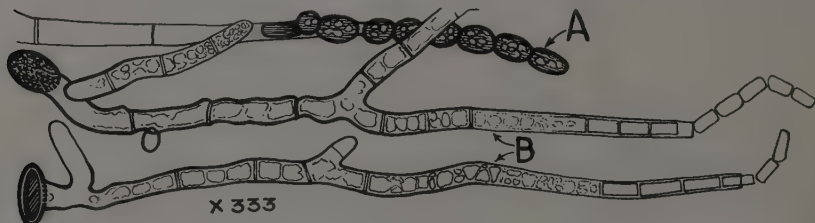


Figure 3. Mycelium and sporophores of the pineapple disease fungus, *Ceratostomella paradoxa*, showing the method of producing macrospores (A) and microspores (B). After Cobb (14).

tested by growing them on a medium of one part soil and one part sand to which 50 grams of cornmeal per 1000 grams of soil and sand were added. This soil-sand-cornmeal medium, hereafter designated as SSC medium, was well mixed and moistened with water, after which several two-quart Mason jars were partially filled with the medium and steamed in the autoclave at 20 lbs. pressure for two hours. After inoculation each isolate was allowed to grow in the SSC medium for seven days during which time the jars were shaken daily to facilitate the spread of the mycelium throughout the medium. Short cane cuttings were then placed in the jars and examined after seven days. An isolate, *Ceratostomella paradoxa*, which caused a rotting of the short cuttings after one week, was selected as the test organism.

The fungus *Ceratostomella paradoxa* enters the cuttings through the cut ends and spreads rapidly through the parenchymatous tissues and first causes a reddening of the tissues, which remain firm for a time. This is followed by a breaking down of the parenchyma and the production of micro- and macrospores (Figure 3). The fibrovascular bundles are not disintegrated (Figure 4). The macrospores are thick-walled and dark in color and are produced in great masses which give the affected cuttings a black or sooty appearance when split longitudinally. The microspores are smaller, thinner-walled, and more transparent than the macrospores. According to Chi (10), the microspores germinate in culture in five to seven hours as compared to 12 to 15 hours for the macrospores. He reports the optimum temperature for the growth of the fungus at 28° C., with a range from 10° to 34° C. He also gives the range of pH for the growth of the fungus as being between pH 3 and 9, with the most suitable range between pH 5 and 7.

**METHODS FOR THE EVALUATION OF FUNGICIDES** 1. *The thread technique.* Since it was desirable to test many fungicides in the laboratory, methods for bioassay of fungicides were reviewed (26,27,28,35,38,40,59,60,61,63) and the evaluation technique reported by Forsberg (24) was selected.

With this method, short sections of sterile No. 8 thread were placed on potato-dextrose agar in a petri dish and the agar was then inoculated with *Ceratostomella paradoxa*. When overgrown with the fungus, the thread was cut into sections about  $\frac{1}{8}$  to  $\frac{3}{16}$  inch in length, with sterile scissors. These pieces of thread were wetted by dipping into different fungicidal concentrations for a



Figure 4. Pineapple disease produced by inoculating a healthy cane stalk with the fungus, *Ceratostomella paradoxa*. The parenchymatous tissue has completely broken down leaving the vascular bundles intact. Photograph by Lyon, 1915.

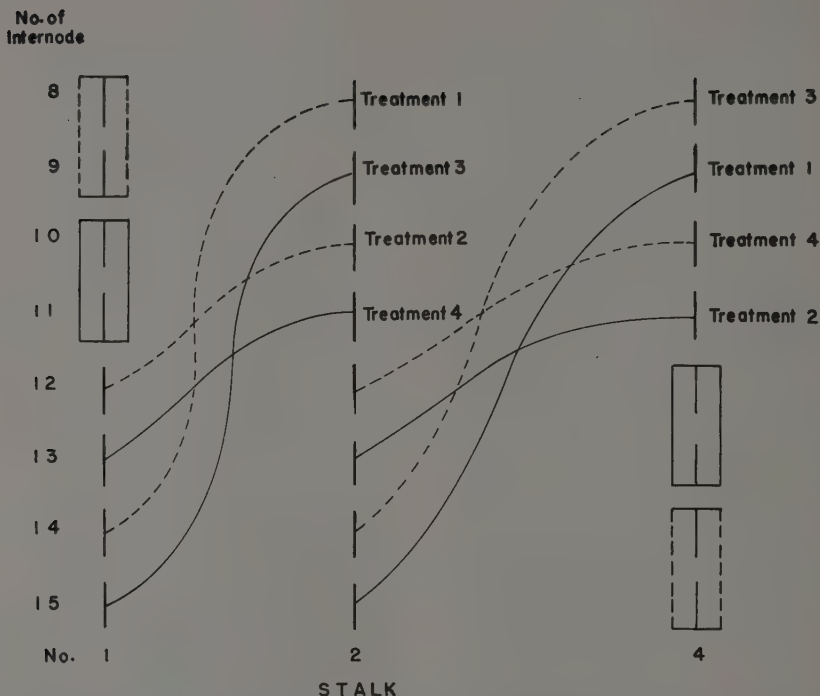


Figure 5. Method used in selecting internodes for fungicidal treatments. Broken lines indicate cuttings examined after one week; solid lines, cuttings examined after two weeks. Boxed-in cuttings are checks. Cutting connected by lines (solid or broken) represent those identically treated and examined at the same time.

few seconds, then blotted on filter paper and planted on potato-dextrose agar in a petri dish. Where no growth appeared from a thread thus dipped, the fungicide was considered effective at that concentration. This method was simple and rapid. Since *C. paradoxa* mycelium grows very rapidly, results were obtained within 24 to 48 hours.

**2. The Soil-sand-cornmeal inoculum technique.** Because it was not practical to test a large number of fungicides in the field it was necessary to use a laboratory evaluation technique in which sugar cane cuttings were used in order to observe whether there was any toxic effect of different concentrations of the fungicides on such cuttings. For this purpose a SSC medium was made up and sterilized in

several two-quart Mason jars, inoculated with the test organism and incubated for one week as previously described for preliminary testing of isolates. This will hereafter be designated as SSC inoculum.

Fungicides were then made up in four dilutions, and four one-eye cuttings were immersed in each concentration of the fungicides for a few seconds. Eight non-treated cuttings used as checks were placed adjacent to treatments in shallow pans. After treating the one-eye cuttings, the SSC inoculum from three to four jars was emptied on a heavy paper, thoroughly mixed and placed in each shallow pan covering the treated and untreated cuttings. Half of these cuttings were then examined after seven days and the remaining half after 14 days (Figure 5).



Three stalks of cane were used to supply the cuttings for each treatment. So that the cuttings for each fungicide would be of the same physiological age, eight cuttings were taken from each stalk. The uppermost cutting sufficiently mature for these studies was selected by counting eight leaves down from the

spindle and including the node to which the eighth leaf was attached. Eight one-eye cuttings were then taken successively down the stalk to include internode No. 15. To reduce variation due to the differences in stalks and age of internode, the arrangement illustrated in Figure 5 was adopted.

## EXPERIMENTAL RESULTS

### THE THREAD TECHNIQUE

Results from the thread technique for the evaluation of fungicides are given in Table 1 and are compared with those obtained from the SSC inoculum technique.

The use of the thread technique for the evaluation of phenyl mercuric acetate, pyridylmercuric acetate, pyridylmercuric chloride and Ceresan against *Ceratostomella paradoxa* is shown in Figure 6. The number "1" on each plate designates the highest concentration of the fungicide and the successively higher numbers indicate a series of dilutions in which each higher number indicates a concentration half that of the next lower number.

Thus the numbers for phenyl mercuric acetate represent a series of dilutions from eight quarts per 100 gallons of water for number 1 to  $\frac{1}{8}$  pint per 100 gallons of water for number 8. In this series the lowest concentration that prevented growth of the fungus was one pint per 100 gallons of water (No. 5).

Numbers on the plates labeled pyridylmercuric acetate and pyridylmercuric chloride represent two ounces per gallon of water for number 1 to  $\frac{1}{64}$  ounce per gallon for number 8. Pyridylmercuric acetate at a dilution of  $\frac{1}{16}$  ounce per gallon of water prevented growth (No. 6), although a little growth appeared on one plate for a dilution of  $\frac{1}{8}$  ounce per gallon of water (No. 5). Pyridylmercuric chloride at  $\frac{1}{4}$  ounce per gallon of water

(No. 4) was effective in preventing growth.

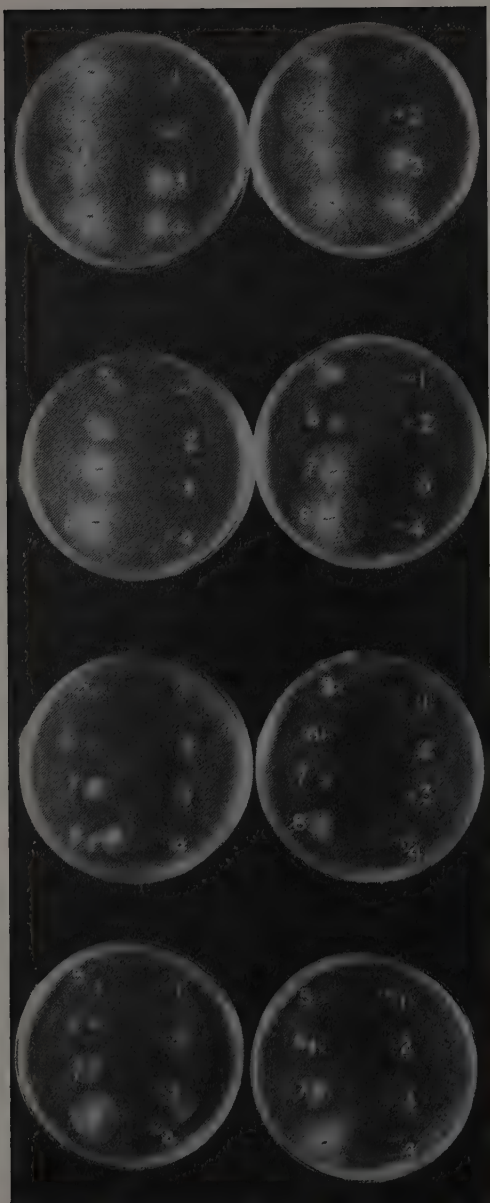
For Ceresan, number 1 represents a dilution of four ounces per gallon of water, while number 8 represents a dilution of  $\frac{1}{32}$  ounce per gallon of water. Ceresan was not effective in preventing the growth of *C. paradoxa* at these dilutions, although there appeared to be some effect at two ounces and four ounces per gallon of water (Nos. 2 and 1).

**THE SOIL-SAND-CORNMEAL INOCULUM TECHNIQUE** The results for those fungicides which were most effective in the control of *Ceratostomella paradoxa* are given in Table 1.

A comparison of the effectiveness of phenyl mercuric acetate with Ceresan in the SSC inoculum after a one week period is shown in Figures 7 and 8.

Phenyl mercuric acetate, one pint per 100 gallons of water, protected cuttings in the SSC inoculum for one week (Figure 7). All cuttings treated with phenyl mercuric acetate including  $\frac{1}{2}$  pint per 100 gallons of water had germinated. The checks which were dipped in water only were completely rotted by *Ceratostomella paradoxa*. Tests with phenyl mercuric acetate treated cuttings in SSC inoculum for two weeks gave good control of rotting by *C. paradoxa* at the rate of one quart per 100 gallons of water.

As previously mentioned, Ceresan was



CERESAN

PYRIDYLMERCURIC CHLORIDE

PYRIDYLMERCURIC ACETATE

PHENYL MERCURIC ACETATE

Figure 6. Use of thread technique for evaluation of fungicides against the fungus, *Ceratostomella paradoxa*. No fungus growth was obtained for phenyl mercuric acetate, diluted one pint per 100 gallons (No. 5); no growth for pyridylmercuric acetate diluted 1/16 ounce per gallon water (No. 6); no growth for pyridylmercuric chloride at 1/4 ounce per gallon of water (No. 4); while Ceresan gave some control at four and two ounces per gallon of water with this technique (Nos. 1 and 2).

TABLE 1

Comparison of the thread technique versus the soil-sand-cornmeal inoculum technique for evaluating the effectiveness of fungicides against *Ceratostomella paradoxa*.

Fungicide	Thread technique	SSC inoculum technique†	
	*Conc. of fungicide that prevented growth for 40 hours**	Conc. of fungicide which prevented rotting for: one week	two weeks
Aretan.....	4	4	0††
Ceresan.....	0	2	4
Copper 8-hydroxy-quinolate.....	$\frac{1}{2}$	$\frac{1}{4}$ & $\frac{1}{2}$ *†	2
Dow 9B.....	2	2	4
Fungicide 1124.....	2	4	0
Lignasan.....	$\frac{1}{8}$	$\frac{1}{4}$ & $\frac{1}{2}$	1
Phenyl mercuric acetate.....	1 pt.	1 pt.	1 qt.
Phygon.....	2	2 (sl)‡	0
Puratized 111-5.....	1 qt.	1 qt.	4 qt.
Puratized Agricultural Spray.....	4 qt.	2 qt.	8 qt.
Puratized GG.....	2 qt.	4 qt.	0
Pyridylmercuric acetate.....	$\frac{1}{16}$	$\frac{1}{16}$	$\frac{1}{8}$ & $\frac{1}{4}$
Pyridylmercuric chloride.....	$\frac{1}{4}$	Lower limits not determined	
Seedox (wettable).....	1	2 (sl)	0
Semesan.....	2	2	4
Sodium pentachlorophenate.....	1	$\frac{1}{2}$ & 1	1 (sl)
Thiosan.....	1	4 (sl)	0

The results for fungicides which were not effective in the control of *Ceratostomella paradoxa* were not included in the above table.

\* Figures represent ounces per gallon of water unless otherwise designated. Liquid fungicides are given in pints and quarts per 100 gallons of water.

\*\* The fungus from the lower concentrations grew over the plate after 40 hours and further readings were not made.

† SSC inoculum consists of one part soil to one part sand plus 5% cornmeal and inoculated with *Ceratostomella paradoxa*.

†† "O" represents no control.

\*† Where two rates are given they indicate that the lower concentration was effective with one test but was not effective for the trial represented by the higher figure.

‡ The letters "sl" indicate that there was only a slight amount of protection of cuttings at this time.

recommended to the plantation at the rate of 1 per cent for the treatment of sugar cane cuttings in 1939 after repeated field testing. In the SSC inoculum, which proved to be a very severe test, control of rotting of cuttings after one week was shown for cuttings treated with Ceresan at the rate of two ounces per gallon of water (Figure 8). Cuttings treated with Ceresan at the rates of  $\frac{1}{2}$  and one ounce per gallon of water did not germinate; when treated with Ceresan at the rates of two and four ounces per gallon of water, they germinated. The checks were completely rotted after one week.

Cuttings examined two weeks after treatment with Ceresan showed that the fungus, *Ceratostomella paradoxa*, had completely penetrated most of the cuttings. Cuttings treated with Ceresan at the rate of four ounces per gallon of

water were only partially rotted.

The results of field studies showed that considerable rotting of cuttings occurred after treatment with fungicides applied immediately following the hot-water treatment (50° C. for 30 minutes). To learn whether or not more protection could be obtained if the cuttings were allowed to dry for a period of time between the hot-water treatment and the application of the fungicide, a laboratory test was made with several intervals of drying before fungicidal treatment. Cuttings given the hot-water treatment were allowed to dry for one, 15, 30, and 60 minutes and then treated with phenyl mercuric acetate at a concentration of one quart per 100 gallons of water. These cuttings were placed in the SSC inoculum and examined two weeks later, at which time the fungus had almost entirely

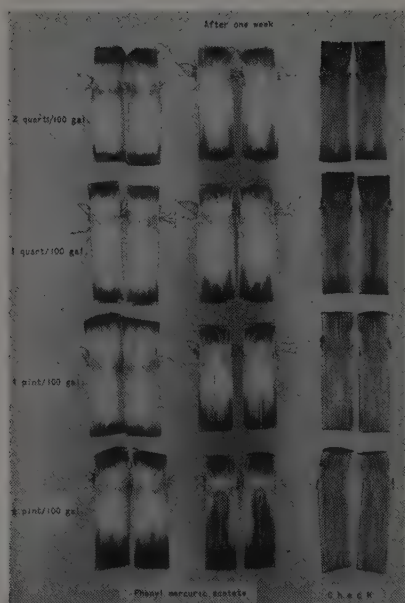
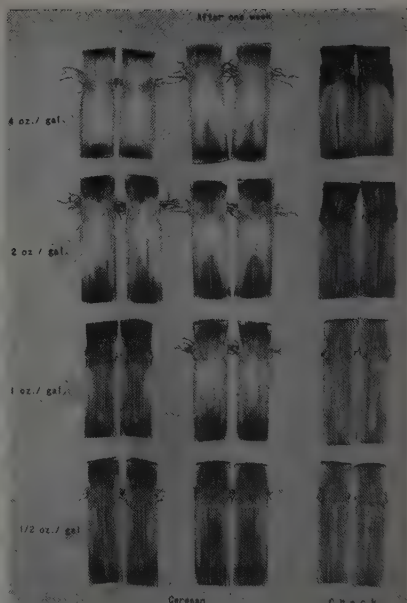


Figure 7. One-eye cuttings non-treated and treated with different concentrations of phenyl mercuric acetate and placed in soil-sand-cornmeal inoculated with *Ceratostomella paradoxa*. Checks were completely rotted. Rotting in treated series is greatest with concentration of  $\frac{1}{2}$  pint per 100 gallons of water.

Figure 8. Note freedom from rotting and germination of one-eye cuttings treated with higher concentrations of Ceresan in contrast with rotting and lack of germination with treatments at lower concentrations and no treatments.



penetrated all the cuttings. However, there was good germination of cuttings treated with phenyl mercuric acetate when compared to the checks, which were completely rotted (Figure 9).

A laboratory test was then made in which phenyl mercuric acetate was added to the water during the hot-water treatment of cuttings at concentrations of  $\frac{1}{2}$  pint, one pint, and one quart per 100 gallons of water. The treated cuttings were incubated in the SSC inoculum. Excellent growth of shoots and protection of cuttings were obtained with these treatments after two weeks (Figure 10). Cuttings dipped in phenyl mercuric acetate at a concentration of one quart per 100 gallons of water and not given the hot-water treatment also gave good protection from rotting after two weeks (Figure 10) as compared to treatment

with the fungicide following the hot-water treatment (Figure 9).

The comparative protection from phenyl mercuric acetate on cuttings when added to and after the hot-water treatment, phenyl mercuric acetate dip alone, and Ceresan dip at a concentration of 1.5 per cent is shown in Figure 11. These cuttings were incubated in SSC inoculum for two weeks.

It was previously pointed out that in other sugar cane growing countries the organic mercurial fungicides are the most effective in protecting sugar cane cuttings from rotting by *Ceratostomella paradoxa*. From the results of laboratory studies it is believed the superiority of these fungicides, aside from the toxic effect of the mercury, is the absorption of the mercurial solution into the ends of the cuttings. When the fungicide is



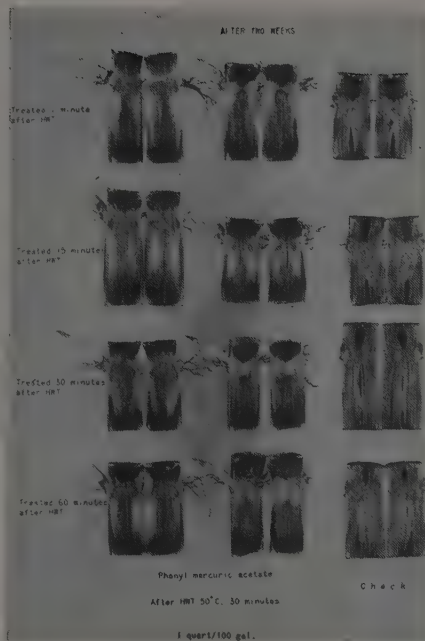
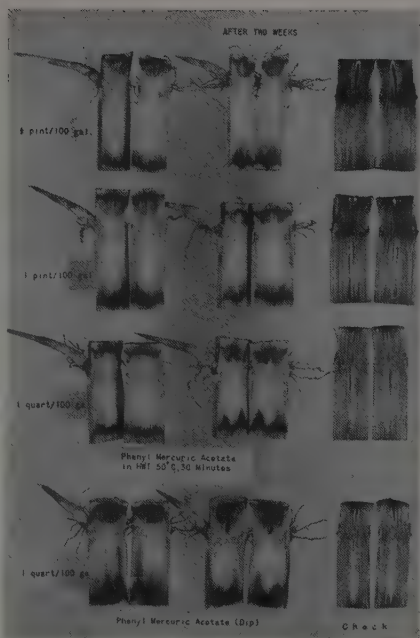


Figure 9. Cuttings given hot-water treatment followed by treatment with phenyl mercuric acetate were badly rotted. Even though buds germinated, shoots would be retarded in growth.

Figure 10. Good protection from rotting was obtained where phenyl mercuric acetate was added to hot-water treatment. Phenyl mercuric acetate at one quart per 100 gallons water also gave protection from rotting when cuttings were dipped without hot-water treatment.



absorbed into the tissue at the ends of the cuttings it is more persistent than when it merely adheres to the ends of the cuttings.

The mercury fungicides kill the ends of the cuttings, leaving a light-colored area with an irregular red band separating this area of dead cells from the normal tissue. The red band produced at the furthest point of penetration by the fungicide is very similar in color to the

red color produced by the penetration of the cells by *C. paradoxa*. However, isolations made from these areas have been free from fungus growth.

It is evident from Figure 10 that phenyl mercuric acetate added in the hot-water treatment has been absorbed to a greater degree and has given more protection from rotting than has the hot-water treatment followed by dipping into the fungicidal solution (Figure 9).

## FIELD CONTROL OF PINEAPPLE DISEASE

The fungicides which, by the evaluation techniques in the laboratory, protected the cuttings best for one and two weeks were tested in the field. Three-eye cuttings were used. They have the advantage over one- or two-eye cuttings since the end nodes act as barriers to

rotting organisms. This allows the center bud more time to germinate and become established before it is weakened by toxins given off by the rotting organism.

Fungicidal treatments were randomized in replicated blocks. Some of the tests were in split plots for inoculation



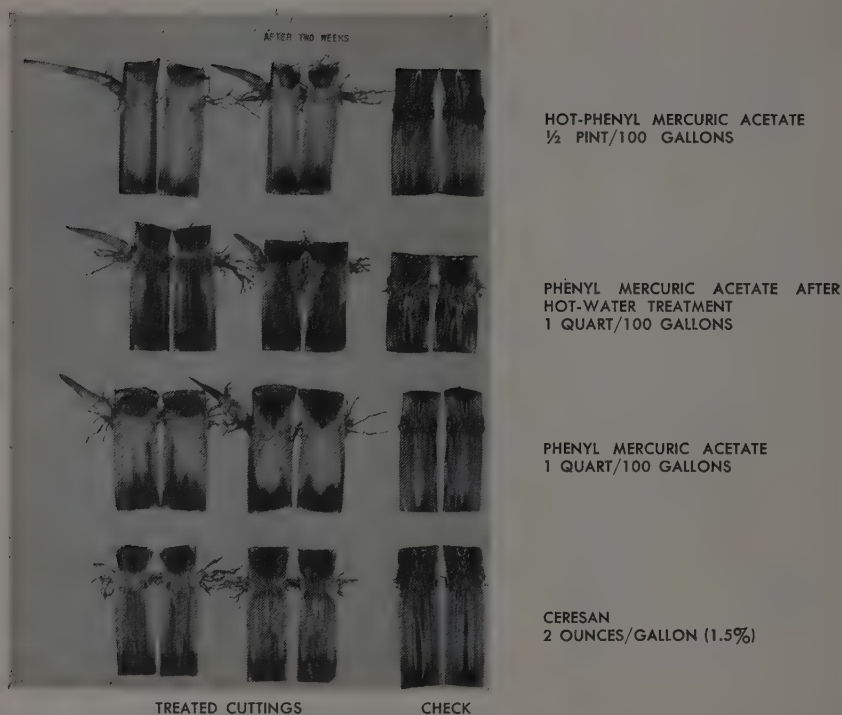


Figure 11. Comparative protection of sugar cane cuttings treated with phenyl mercuric acetate with and after the hot-water treatment; phenyl mercuric acetate alone; and Ceresan. Note the good protection from the first and third treatments.

with the test organism and/or section of the stalk from which the cutting was taken, e.g., the top cutting and the next lower cutting and in some tests, a third and fourth cutting. In planting, the buds were turned to the side and the cuttings covered to a depth of about two inches.

**PRELIMINARY TEST OF FUNGICIDES** A field test was made at Kailua, Oahu, with the commercial variety 32-8560 to compare those fungicides that had most effectively protected cuttings in the laboratory, namely, pyridylmercuric acetate, pyridylmercuric chloride and phenyl mercuric acetate. Also included were Ceresan, the fungicide being used by the plantations, and Aretan, Phygon and Semesan which had

shown promise in other sugar cane growing countries even though they were inferior in laboratory tests.

Ceresan, pyridylmercuric acetate, pyridylmercuric chloride, Aretan, Phygon and Semesan were used for treating cuttings at the rates of  $\frac{1}{2}$  and one ounce per gallon of water. Phenyl mercuric acetate was tested at concentrations of one and two quarts per 100 gallons of water. Cuttings were treated by dipping the ends into the different concentrations of each fungicide.

The planting material consisted of two three-eye cuttings taken from each stalk. Each plot was planted with 10 cuttings, which included five top cuttings and five cuttings taken from the portion of the stalk immediately below the top cutting.

TABLE 2

Percentage of germination and rotting of sugar cane cuttings inoculated with *Ceratostomella paradoxa* (n=64)\*

Treatment	Percentage of Germination	Percentage of Rotting
Not inoculated.....	45.4	33
Inoculated.....	30.0	80
	**L.S.D. .05=3.3%	L.S.D. .05=5.0%

\* "n" = The number of figures averaged to give each percentage figure in the table.

\*\* L.S.D. = Least significant differences, e.g.  $P = .05$

Half of the cuttings were inoculated by dipping their ends into a spore suspension of *Ceratostomella paradoxa* just before planting. The cuttings were planted during a period of dry weather and no irrigation water was applied until a week after planting.<sup>5</sup> After the first irrigation, water was applied as needed so that moisture was not a limiting factor for normal growth.

Germination counts were made at weekly intervals. The final count was made seven weeks after planting, when the cuttings were dug up and all shoots counted. The cuttings were also split and examined for amount of rotting. Notes on vigor were taken just prior to digging the cuttings in order to determine the relative growth of the shoots for the different treatments. Statistical analyses were made for germination and rotting for the different treatments.

The effects on germination and rotting from the inoculation of cuttings with *Ceratostomella paradoxa* were highly significant, as shown in Table 2.

Germination from the inoculated cut-

tings was definitely poorer and the amount of rotting greater, indicating that dipping the ends of the cuttings into a spore suspension of *Ceratostomella paradoxa* was a satisfactory method for the field inoculation of cuttings.

Table 3 shows the effect on germination for the different fungicides for cuttings inoculated and not inoculated.

Each figure in Table 3 represents an average of the results for fungicides used at concentrations of one and  $\frac{1}{2}$  ounces per gallon of water, except for phenyl mercuric acetate which was used at concentrations of one and two quarts per 100 gallons of water and the control which was dipped in water only.

With the non-inoculated cuttings, all fungicides were equally effective and better than the check. The rotting organism *Ceratostomella paradoxa* must be present in order to permit evaluating the protective value of the fungicides.

The following differences in germination of inoculated cuttings were evident:

a. Pyridylmercuric acetate and pyridylmercuric chloride were definitely

TABLE 3

Percentage of germination of sugar cane cuttings inoculated and non-inoculated with *Ceratostomella paradoxa* and treated with different fungicides (n=8)

Fungicide	Percentage of Germination	
	Inoculated	Not Inoculated
Pyridylmercuric chloride.....	52.1	49.6
Pyridylmercuric acetate.....	49.2	48.7
Phenyl mercuric acetate.....	37.5	51.3
Aretan.....	30.8	45.8
Ceresan.....	21.7	49.6
Control.....	18.7	25.4
Semesan.....	17.1	46.7
Phygon.....	10.8	46.3
	L.S.D. .05=10%	

<sup>5</sup> The Genetics Department of the Experiment Station, HSPA had shown that treatment with fungicides was especially beneficial when irrigation was delayed during dry weather.

better than all other fungicides at the concentrations used in this test.

b. Phenyl mercuric acetate was better than the check, Ceresan, Phygon and Semesan but was not shown to be better than Aretan on the basis of germination.

c. Ceresan, Phygon and Semesan were no better than the control.

These results show some correlation with those obtained in laboratory studies using the SSC inoculum, except for Aretan which showed up better on the basis of germination under the field studies than in the laboratory tests (Table 1). However, as shown in Tables 4 and 5, cuttings treated with Aretan were badly rotted and the shoots were less vigorous than were cuttings treated with fungicides which gave more protection.

The amount of rotting by *Ceratostomella paradoxa* for cuttings treated with the different fungicides is shown in Table 4.

These results show that pyridylmercuric acetate, pyridylmercuric chloride, and phenyl mercuric acetate protected cuttings most effectively. Aretan gave the least protection from rotting of any

TABLE 4  
Average percentage of rotting of sugar cane cuttings treated and non-treated with *Ceratostomella paradoxa*, and treated with different fungicides (n=16).

Fungicides	Percentage of totally rotted cuttings
Pyridylmercuric acetate.....	26
Pyridylmercuric chloride.....	37
Phenyl mercuric acetate.....	39
Phygon.....	59
Ceresan.....	62
Semesan.....	67
Aretan.....	70
Control.....	90
L.S.D. .05=9%	

fungicide in this test, which is in agreement with the results obtained in laboratory studies (Table 1).

Notes on vigor of shoots taken at the termination of the test, on plots inoculated and not inoculated, show the effect of the fungus on the growth of the shoots (Table 5). When all or most of the cuttings in a plot were completely rotted with *Ceratostomella paradoxa* the shoots were nearly always less vigorous than the shoots in a plot with only a few of the cuttings completely rotted.

The fungicides pyridylmercuric acetate, pyridylmercuric chloride and phenyl

TABLE 5

The vigor of sugar cane shoots for cuttings inoculated and non-inoculated with *Ceratostomella paradoxa* and treated with different fungicides.

Fungicide	Concentration in ounces per gallon	Vigor**		No. of Cuttings Completely Rotted†	
		Inoc.††	Not Inoc.	Inoc.	Not Inoc.
Ceresan.....	1/2	2.3	3.3	40	14
	1	2.8	3.5	36	9
Aretan.....	1/2	1.5	3.0	37	18
	1	2.0	3.3	37	20
Phygon.....	1/2	1.3	3.8	40	8
	1	1.5	3.3	40	6
Semesan.....	1/2	1.5	3.5	39	15
	1	2.0	3.5	38	15
Pyridylmercuric acetate.....	1/2	3.5	3.3	22	9
	1	3.5	4.0	12	6
Pyridylmercuric chloride.....	1/2	3.0	3.8	28	4
	1	4.3	3.5	20	6
Phenyl mercuric acetate*.....	1	3.0	3.5	27	10
	2	3.3	3.5	22	5
Control.....		2.5	2.0	40	32
		1.8	1.8	40	32

\* The concentration for phenyl mercuric acetate is given in quarts per 100 gallons of water.

\*\* Vigor is given on a basis of 1 to 5 where 1 represents very poor growth, 3 average, and 5 excellent growth of shoots

† Each figure represents the number of cuttings totally rotted from a total of 40 cuttings planted.

†† Cuttings inoculated with spores of *Ceratostomella paradoxa*.

mercuric acetate are the only ones which, in the presence of the inoculum, gave as little rotting, and as good vigor as treated non-inoculated cuttings (Table 5).

**TESTS FOR OPTIMUM CONCENTRATIONS** Since pyridylmercuric acetate and pyridylmercuric chloride were shown to control rotting by *Ceratostomella paradoxa* in the field, a test was designed wherein a series of dilutions was used ranging from one to  $\frac{1}{32}$  ounce per gallon of water for these fungicides. The concentrations for phenyl mercuric acetate in this test were one quart and one pint per 100 gallons of water, since two quarts per 100 gallons of water had shown no increase in germination over one quart per 100 gallons in earlier field tests. Other fungicides were included which showed some protection of cuttings when treated and incubated in the SSC inoculum in the laboratory. The hot-water treatment (50° C. for 30 minutes) was also included in this test. The cuttings in half the plots were inoculated by dipping the ends of the cuttings into a spore suspension of *Ceratostomella paradoxa*. The test was located at Pearl City, Oahu, planted with the variety 32-8560 and immediately irrigated.

Weather conditions were favorable for

germination and subsequent growth of the cane shoots so that shoot roots became established before the shoots were affected by the penetration of the fungus. As a result of the favorable conditions for germination the differences in percentage germination were small (Appendix, Table II). Similar results for percentage of germination of the variety 38-2915 were obtained for treated cuttings in a test at Oahu Sugar Company when the cuttings were not inoculated with *Ceratostomella paradoxa* (Appendix, Table III). However, the differences in the percentage of rotting at the termination of the test with the variety 32-8560 eight weeks after planting give a good indication of the protection from the different treatments as shown in Table 6.

Percentage of rotting in the control was 47.5 per cent (Appendix, Table II).

The minimum concentration of pyridylmercuric acetate which protected cuttings in this test was  $\frac{1}{16}$  ounce per gallon of water, which correlates with the results obtained for this fungicide with the thread technique and in SSC inoculum after one week in the laboratory (Table 1). The correlation for pyridylmercuric chloride was not as good but the results with the thread technique

TABLE 6  
Percentage of rotting of sugar cane cuttings inoculated and non-inoculated with *Ceratostomella paradoxa* and treated with different concentrations of fungicides (n=6).

Fungicide	Concentration*	Percentage of Rotting of Cuttings	
		Inoculated	Not Inoculated
Pyridylmercuric acetate.....	1	1.7	0.0
" ".....	$\frac{1}{2}$	1.7	1.7
" ".....	$\frac{1}{4}$	5.0	5.0
" ".....	$\frac{1}{8}$	8.3	3.3
" ".....	$\frac{1}{16}$	13.3	1.7
" ".....	$\frac{1}{32}$	35.0	1.7
Pyridylmercuric chloride.....	1	5.0	0.0
" ".....	$\frac{1}{2}$	6.7	0.0
" ".....	$\frac{1}{4}$	6.7	1.7
" ".....	$\frac{1}{8}$	8.3	0.0
" ".....	$\frac{1}{16}$	23.3	0.0
" ".....	$\frac{1}{32}$	35.0	8.3
Ceresan.....	1%	26.7	1.7
Phenyl mercuric acetate.....	1 qt.	15.0	3.3
" ".....	1 pt.	23.3	0.0

L.S.D. 14.8%

\* All concentrations are given in ounces per gallon of water except for Ceresan used at 1 per cent and phenyl mercuric acetate used at the rate of one quart and one pint per 100 gallons of water.

are still close enough to indicate the relative value of the fungicide by this technique. Since the other fungicides were not tested in so large a number of dilutions, the comparison with laboratory results is less reliable (Appendix, Table II).

It is again evident from the results in Table 6 that it would be more difficult to evaluate these fungicides if half the cuttings had not been inoculated with *Ceratostomella paradoxa*. The greater the difference in percentage of rotting between the check and treated cuttings the greater the insurance value of the best fungicide when conditions are unfavorable for germination.

The percentage of germination for the variety 32-8560 was increased by the hot-water treatment (50° C. for 30 minutes) over most other treatments (Appendix, Table II), while for the variety 38-2915 the hot-water treatment gave a lower percentage of germination than the check (Appendix, Table III). The percentage of rotting for these varieties receiving the hot-water treatment was high in comparison with other treatments. A higher resistance to the penetration of *Ceratostomella paradoxa* through the cuttings of the variety 32-8560 is indicated as compared to the variety 38-2915, thus allowing the shoots of the variety 32-8560 to become established before they are killed or retarded by the fungus.

**PHENYL MERCURIC ACETATE AND HOT-WATER TREATMENTS** Since the hot-water treatment stimulated germination of the variety 32-8560 but was deleterious to the variety 38-2915 in earlier field experiments, a field test was made at Kahuku Plantation Company to obtain further information. Since the variety 37-1933 is an important commercial variety in Hawaii it was also included in this test. The following

treatments were used: (1) control; (2) the hot-water treatment (50° C. for 30 minutes); (3) phenyl mercuric acetate dip at a dilution of one quart per 100 gallons; (4) hot-water treatment followed by dipping the cuttings into phenyl mercuric acetate. The varieties 32-8560 and 38-2915 were taken from adjacent areas in the same field while the variety 37-1933 was obtained from a near-by field. All varieties were about 10 months old when cut for planting. The cuttings of each variety were divided into two lots: the two top three-eye cuttings were designated as top cuttings and two three-eye cuttings taken from that portion of the stalk immediately below the two top cuttings were designated as bottom cuttings.

The cuttings in alternate blocks were inoculated with *Ceratostomella paradoxa* by dipping the ends into a spore suspension of the fungus.

The test was divided so that half the plots were irrigated immediately while the remainder were irrigated 11 days after planting. The irrigation differential was to note the effect of delayed irrigation on the different treatments and to determine the best treatment to use on unirrigated plantations where rain might not fall immediately after planting.

Since the analyses of variance of these results were difficult to interpret when the figures for percentage of germination and rotting for cuttings inoculated with *Ceratostomella paradoxa* were included with those for non-inoculated cuttings, these two sets of data have been analysed separately.

*1. Effects of treatment on the germination of inoculated cuttings.* Sugar cane cuttings treated with phenyl mercuric acetate gave high percentages of germination in the treatments in this test when compared with the hot-water treatment (50° C. for 30 minutes) and phenyl mercuric acetate after the hot-water treatment.



TABLE 7

Average percentage of germination of cuttings of three varieties of sugar cane inoculated with *Ceratostomella paradoxa* and treated in different ways (n=36).

Treatment	Percentage of Germination
Control.....	46.6
Hot-water treatment.....	47.2
Phenyl mercuric acetate.....	74.0
Phenyl mercuric acetate after hot-water treatment.....	65.2
	L.S.D. .05=6.4%

The results for these treatments are shown in Table 7.

From the averages in Table 7 it is evident that the effectiveness of phenyl mercuric acetate is reduced when it is applied to the cuttings after they have been treated with hot-water.

The hot-water treatment alone did not result in any increase in percentage germination.

A definite effect of treatment with phenyl mercuric acetate on germination

The protective effect of phenyl mercuric acetate applied to cuttings after they were treated with hot-water was less when irrigation was delayed than that of phenyl mercuric acetate alone. (See Table 8).

Differences in percentage of germination of sugar cane varieties from treatments with phenyl mercuric acetate and hot-water are given in Table 9.

The largest percentage increase in germination from treatment with phenyl

TABLE 8

Average percentage germination of cuttings of three varieties inoculated with *Ceratostomella paradoxa*, treated in different ways and irrigated immediately after planting and 11 days after planting (n=18).

Treatment	Irrigated	
	Immediately	After 11 days
	Percentage of Germination	
Control.....	59.4	33.7
Hot-water treatment.....	60.4	33.9
Phenyl mercuric acetate.....	76.5	71.5
Phenyl mercuric acetate after hot-water treatment.....	72.2	58.1
	L.S.D. .05=7.5%	L.S.D. .05=8.0%

was observed when irrigation was delayed for 11 days as compared to the percentage germination for cuttings irrigated immediately. This effect is shown by the results in Table 8.

The percentage of germination was increased by phenyl mercuric acetate both when irrigation was delayed and when water was supplied immediately after planting, although the beneficial effect was greater when irrigation was delayed.

mercuric acetate over the check was for the variety 38-2915, while the variety 37-1933 did not seem to respond as well to treatment with phenyl mercuric acetate. The variety 32-8560 seems more resistant to the factors which tend to decrease germination.

Effect of treatment with phenyl mercuric acetate for varieties irrigated at different intervals after planting is shown by the differences for averages from the

TABLE 9

The percentage germination of cuttings of three varieties of sugar cane inoculated with *Ceratostomella paradoxa* and then treated in different ways and planted in the field (n=12).

Treatment	Variety		
	32-8560	37-1933	38-2915
Control.....	60.0	40.0	39.8
Hot-water treatment.....	54.8	48.9	37.8
Phenyl mercuric acetate.....	83.6	66.2	77.2
Phenyl mercuric acetate after hot-water treatment.....	75.6	54.2	65.9
		L.S.D. .05=12.8%	

TABLE 10

The percentage germination of cuttings of sugar cane varieties inoculated with *Ceratostomella paradoxa* and treated with phenyl mercuric acetate and with hot-water and irrigated immediately and after 11 days (n=12).

Average for	Irrigated					
	Immediately			After 11 Days		
	32-8560	37-1933	38-2915	32-8560	37-1933	38-2915
Control+Hot-water treatment.	71.7	58.6	49.5	43.1	30.3	28.1
Phenyl mercuric acetate+ phenyl mercuric acetate after hot-water treatment. . . . .	84.5	66.2	72.5	74.7	49.2	70.6
Difference between means . . . . .	12.8	7.6	23.0	31.6	18.9	42.5

results for treatments with and without phenyl mercuric acetate in Table 10.

The beneficial effects of phenyl mercuric acetate for the different varieties when irrigation was delayed is in relation to these effects where irrigation was immediate. The differences for average percentage germination between treatments with and without phenyl mercuric acetate are greater when irrigation was delayed. This shows the advantage of treating cuttings with phenyl mercuric acetate when conditions are unfavorable for germination.

TABLE 11

Percentage of germination of all cuttings in the test for stalk sections of three varieties of sugar cane inoculated with *Ceratostomella paradoxa* and irrigated 11 days after planting (n=12)

Variety	Stalk Sections	
	Top Cuttings	Bottom Cuttings
32-8560. . . . .	66.1	51.7
37-1933. . . . .	55.3	24.2
38-2915. . . . .	61.1	37.5
	L.S.D. .05 = 8.8%	

Top cuttings of each of the varieties 32-8560, 37-1933 and 38-2915 gave a higher percentage of germination than the bottom cuttings when irrigation was delayed (Table 11).

The differences in percentage for top

and bottom cuttings show that it is necessary to have the same number of top and bottom cuttings in a plot or to use only one type of cutting for each plot in field experiments in order to avoid the effects of differential germination.

2. *Effects of treatment on the germination of non-inoculated cuttings.* There were no significant effects of fungicides alone or of fungicides with varieties or with stalk sections when cuttings were irrigated immediately. There were some differences, however, when these three factors were separated so that their effects on each other could be studied. The results for their averages are shown in Table 12.

Phenyl mercuric acetate did not affect germination when cuttings were irrigated immediately after planting (Table 12), which is contrary to results for cuttings inoculated with *Ceratostomella paradoxa* (Table 9). It is evident from these results that it is not practical to attempt to evaluate a fungicide such as phenyl mercuric acetate when the rotting organism is not uniformly common.

A definite stimulation of germination for top cuttings of the variety 37-1933 resulted from the hot-water treatment, which was not shown for the bottom

TABLE 12

Percentage of germination of non-inoculated top cuttings and bottom cuttings of sugar cane treated in different ways and irrigated immediately after planting (n=3)

Treatment	Stalk Sections					
	Top Cuttings			Bottom Cuttings		
	32-8560	37-1933	38-2915	32-8560	37-1933	38-2915
Control. . . . .	84.4	68.9	85.6	78.9	68.9	60.0
Hot-water treatment. . . . .	82.2	92.2	74.4	78.9	64.4	63.3
Phenyl mercuric acetate. . . . .	87.8	73.3	84.4	90.0	65.5	63.3
Phenyl mercuric acetate after hot-water treatment. . . . .	94.4	91.1	77.8	85.6	62.2	66.7
	L.S.D. .05 = 13.9%					

cuttings. There was a lower percentage of germination for bottom cuttings of the varieties 37-1933 and 38-2915 than for the majority of top cuttings regardless of treatment.

When irrigation was delayed an increase in percentage of germination from phenyl mercuric acetate was shown (Table 13).

The percentage of germination for cuttings treated with phenyl mercuric acetate after the hot-water treatment was equal to that for cuttings treated with phenyl mercuric acetate alone where the cuttings were not inoculated and irrigation was delayed (Table 13). The phenyl mercuric acetate alone gave a higher percentage of germination for inoculated cuttings with delayed irrigation (Table 8).

A study of the beneficial effect of phenyl mercuric acetate on the germination of non-inoculated cuttings when irrigation was delayed shows that the

TABLE 13  
Average percentage of germination of non-inoculated cuttings of three varieties of sugar cane treated in different ways and irrigated 11 days after planting (n=18).

Treatment	Percentage of Germination
Control.....	63.1
Hot-water treatment.....	66.1
Phenyl mercuric acetate.....	80.6
Phenyl mercuric acetate after hot-water treatment.....	79.8
	L.S.D. .05=6.7%

planting when conditions are unfavorable for rapid germination.

3. *Effects of treatment on the rotting of cuttings.* The results from treatments with phenyl mercuric acetate and hot-water are shown in Table 15 by the averages for cuttings inoculated and not inoculated with *Ceratostomella paradoxa*.

Phenyl mercuric acetate was the most effective treatment for reducing the rotting of sugar cane cuttings inoculated with *Ceratostomella paradoxa*. Treatment with hot water apparently made the cuttings more susceptible to rotting and

TABLE 14  
Percentage of germination for non-inoculated top cuttings and bottom cuttings of three varieties when irrigation was delayed for 11 days after planting (n=9).

Treatment	Stalk Sections	
	Top Cuttings	Bottom Cuttings
Control.....	77.8	48.5
Hot-water treatment.....	82.2	50.0
Phenyl mercuric acetate.....	79.6	81.5
Phenyl mercuric acetate after hot-water treatment.....	85.9	73.7
	L.S.D. .05=9.5%	

increase was due to a higher percentage of germination for bottom cuttings, these being no different from the top cuttings. The averages for these treatments are shown in Table 14.

These results show the importance of treatment with phenyl mercuric acetate if other than top cuttings are used for

phenyl mercuric acetate was unable to counteract this deleterious effect when applied following the hot-water treatment. The differences in percentage rotting are not as large for non-inoculated cuttings as for inoculated cuttings.

The differential effect of treatments with phenyl mercuric acetate in pro-

TABLE 15  
Average percentage of rotting of inoculated and not inoculated cuttings of three varieties of sugar cane treated with phenyl mercuric acetate and hot-water (n=36).

Treatment	Percentage of Rotting for Cuttings	
	Inoculated	Not Inoculated
Control.....	60.0	18.9
Hot-water treatment.....	71.8	34.2
Phenyl mercuric acetate.....	13.7	4.5
Phenyl mercuric acetate after hot-water treatment.....	45.8	9.5
	L.S.D. .05=10.7%	

TABLE 16  
Percentage of rotting of cuttings of three varieties of sugar cane inoculated with *Ceratostomella paradoxa*, treated in different ways and irrigated 11 days after planting (n=6).

Treatment	Percentage of Rotting per Variety		
	32-8560	37-1933	38-2915
Control.....	63.3	96.6	83.3
Hot-water treatment.....	90.0	90.0	91.7
Phenyl mercuric acetate.....	5.0	40.0	20.0
Phenyl mercuric acetate after hot-water treatment.....	38.3	83.3	56.7
L.S.D. .05=17.0%			

testing different varieties from rotting when irrigation was delayed is shown by the results in Table 16.

Phenyl mercuric acetate gave the greatest protection to all varieties against rotting. Phenyl mercuric acetate after the hot-water treatment was much less effective in protecting the cuttings of the variety 37-1933 from rotting than was the same treatment for 32-8560 or 38-2915. The hot-water treatment caused the cuttings of the variety 32-8560 to be

*Ceratostomella paradoxa*, is not evident in these results (Table 17) except for the variety 38-2915.

Treatment with phenyl mercuric acetate was not shown to be effective for controlling rotting of cuttings over the check for the varieties 32-8560 or 37-1933. This shows the danger of drawing conclusions concerning the value of phenyl mercuric acetate for protecting cuttings against rotting when the organism which causes the rotting is not present.

TABLE 17  
Percentage of rotting of non-inoculated cuttings of three varieties of sugar cane, treated in different ways and irrigated immediately after planting (n=6).

Treatment	Percentage of Rotting per Variety		
	32-8560	37-1933	38-2915
Control.....	3.3	13.3	35.0
Hot-water treatment.....	18.3	26.7	56.7
Phenyl mercuric acetate.....	5.0	8.3	6.7
Phenyl mercuric acetate after hot-water treatment.....	6.7	8.3	26.7
L.S.D. .05=14.7%			

more susceptible to the rotting organism, *Ceratostomella paradoxa*.

The effect of phenyl mercuric acetate and hot-water on the rotting of cuttings for the different varieties when they were not inoculated and irrigated immediately after planting is shown in Table 17.

Failure of phenyl mercuric acetate to protect cuttings from rotting after the hot-water treatment, which was shown when the cuttings were inoculated with

The effect of phenyl mercuric acetate and hot water on the rotting of non-inoculated stalk sections of different varieties is shown in Table 18 for data taken where the irrigation of cuttings was delayed.

Protection of top cuttings of all varieties from rotting by treatment with phenyl mercuric acetate was similar to that of the bottom cuttings. In general, there was more rotting of bottom cut-

TABLE 18  
Percentage of rotting of non-inoculated top cuttings and bottom cuttings of three varieties of sugar cane, treated in different ways and irrigated 11 days after planting (n=3).

Treatment	Top Cuttings			Bottom Cuttings		
	32-8560	37-1933	38-2915	32-8560	37-1933	38-2915
Control.....	36.7	96.7	66.7	90.0	96.7	100.0
Hot-water treatment.....	93.3	80.0	83.3	86.7	100.0	100.0
Phenyl mercuric acetate.....	3.3	33.3	20.0	6.7	46.7	20.0
Phenyl mercuric acetate after hot-water treatment.....	33.3	73.3	50.0	43.3	93.3	100.0
L.S.D. .05=24.1%						



tings for the different varieties. All cuttings of the varieties 32-8560 and 38-2915 were effectively protected by treatment with phenyl mercuric acetate alone. The inability of phenyl mercuric acetate to counteract the effect of the

hot-water treatment is plainly indicated. The lower percentage of rotting of the top cuttings of 37-1933 treated with hot water is believed to be due to the stimulation of the buds resulting in faster rate of germination for this variety.

## DISCUSSION

Pineapple disease of sugar cane caused by *Ceratostomella paradoxa* was found to cause most of the rotting of sugar cane cuttings in Hawaii where it has been found on all cane plantations. Under conditions favorable for its development, the pineapple disease organism rapidly penetrates the cuttings and kills the buds or causes rotting of the cutting with consequent production of weak shoots.

There is some evidence that, when buds of sugar cane cuttings germinate, antibiotic substances are produced which delay the penetration of the cuttings by *C. paradoxa*. Whenever cuttings with vigorous shoots are dug up and examined these cuttings are seldom completely rotted, while a high percentage of cuttings which do not germinate quickly are completely rotted. This results in poor stands, especially when fields are planted during periods unfavorable for normal growth, and necessitates costly replanting. Conditions are likely to be unfavorable in fall and winter when the buds are dormant. Low soil temperatures in the winter months, excessively wet or dry conditions, and too deep planting are unfavorable for germination. Results from South Africa, Mauritius, Australia and Hawaii indicate that germination can be improved by treating cuttings with an effective fungicide prior to planting either in excessively dry or wet soil.

The present work was begun in 1949 to find a fungicide that would be more satisfactory for treating sugar cane cuttings than Ceresan which was being used by the plantations. More than 60 fungicides were obtained for testing. The

thread technique proposed by Forsberg (24) and a technique in which one-eye cane cuttings were treated with the fungicides and incubated in a soil-sand-cornmeal medium inoculated with *Ceratostomella paradoxa* proved to be satisfactory for evaluating these fungicides in the laboratory. A comparison was made between the laboratory methods and replicated field testing for evaluating fungicides.

It was demonstrated that in order to evaluate fungicides under field conditions it is essential to inoculate the cuttings with *Ceratostomella paradoxa*. In other words, it is necessary to have the fungus present before determining the fungicidal or fungistatic properties of any fungicide.

Phenyl mercuric acetate at a concentration of one quart per 100 gallons of water was found equal to other fungicides in protecting sugar cane cuttings from rotting by *Ceratostomella paradoxa* in the field, and it is less expensive. It is efficient, economical, easy to apply, and is as permanent as any other fungicide tested. It does not injure the cuttings at the rate recommended for treatment. Furthermore, the 10 per cent aqueous solution does not contain any inert material.

To properly appreciate the protection to cuttings from treatment with phenyl mercuric acetate, it is essential that cuttings be planted under conditions which are sub-optimum for germination. Two such conditions used to illustrate this point were the presence of a large population of the organism and delay in irri-



gation after planting. When both these conditions were present at the same time there was a greater benefit from treatment with phenyl mercuric acetate.

When conditions are favorable for germination, all percentages for germination may be high. While an increase of 15 to 20 per cent in germination may not appreciably affect the yield when the percentage germination is normally above 60 per cent, where such a difference in germination is shown to be the result of a treatment, the same or a greater increase can be expected when conditions are less favorable. A 15 to 20 per cent difference in germination, when the average percentage germination is around 40 per cent, as in the fall and winter months, might mean the difference between finding it necessary to replant or not to replant a field.

The hot-water treatment was included in these tests as a stimulant to bud germination in conjunction with fungicidal protection. The sugar cane varieties 32-8560, 37-1933 and 38-2915 responded differently to the hot-water treatment. There was a good growth response from

the hot-water treatment by 32-8560 and 37-1933 when they were irrigated immediately after planting. Poorer germination was obtained from the variety 38-2915 treated with hot water in comparison with the control in one test, while in a second test it was not different from the control. All varieties should be tested with the hot-water treatment on a moderate scale before too great an amount of planting material (cuttings) is treated for the purpose of stimulating bud germination.

It was shown in laboratory and field studies that phenyl mercuric acetate was less effective when used after the hot-water treatment. However, laboratory tests with phenyl mercuric acetate (10 per cent aqueous solution) at a concentration of a half pint per 100 gallons of water, placed in the water during the hot-water treatment, showed this treatment to be as effective in preventing rotting with *Ceratostomella paradoxa* as cuttings without the hot-water treatment dipped in phenyl mercuric acetate at a concentration of one quart per 100 gallons of water.

## SUMMARY

1. The pineapple disease organism, *Ceratostomella paradoxa* (de Seynes) Dade, is the principal cause of rotting of sugar cane cuttings in Hawaii.

2. The thread technique of Forsberg for the evaluation of fungicides was a rapid and satisfactory method for the preliminary screening of fungicides in the laboratory with the test organism, *C. paradoxa*.

3. Good results were obtained in final laboratory screening of fungicides by immersing one-eye sugar cane cuttings in a series of concentrations of the fungicide and incubating them in pans with a soil-sand-cornmeal medium inoculated with the test organism. This method disclosed any phytotoxic effects of the fungicide on the cuttings.

4. There was good correlation between results from tests with fungicides by the thread technique, the sand-soil-cornmeal inoculum method, and replicated field tests where the sugar cane cuttings were inoculated with *C. paradoxa*. Pyridylmercuric acetate, pyridylmercuric chloride and phenyl mercuric acetate controlled the pineapple disease organism effectively in each of the three tests.

5. Most of the field tests were not satisfactory for the selection of the best fungicide unless the cuttings were first inoculated with the pineapple disease organism.

6. Cuttings were inoculated with the pineapple disease organism by dipping the ends of the cuttings into a spore suspension of *Ceratostomella paradoxa*.

7. Varieties respond differently to treatment with hot water (50° C. for 30 minutes). Germination for the variety 37-1933 with the hot-water treatment was improved, whereas no such response was obtained for the variety 38-2915.

8. Hot-water treatment generally increased the susceptibility of cuttings to rotting with subsequently poor germination.

9. The hot-water treatment should not be used where sugar cane cuttings do not receive sufficient moisture for germination soon after planting.

10. The hot-water treatment of cuttings reduced the protective value of phenyl mercuric acetate when the cuttings were treated with the fungicide after the hot-water treatment.

11. Excellent protection of cuttings from rotting was obtained in the laboratory when phenyl mercuric acetate (10 per cent aqueous solution) was added to the hot-water at the rate of a half pint per 100 gallons of water.

12. Phenyl mercuric acetate has proved equal to any other fungicide in the field and is cheaper at the concentrations which are effective for the protection of cuttings against *Ceratostomella paradoxa*.

13. Phenyl mercuric acetate is recommended for the treatment of sugar cane cuttings at a concentration of one quart to 100 gallons of water for dipping or spraying of cuttings, or a half pint to 100 gallons of water when the fungicide is added in the hot-water treatment.

14. Sugar cane cuttings should be treated with a fungicide when planted under conditions that are adverse for the germination of the buds, especially when the soil is either very wet or very dry.

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# APPENDIX

TABLE I

Fungicides supplied by Chemical Companies for evaluation against *Ceratostomella paradoxa*.

Name	Chemical Compound	Chemical Company
Aretan (Clerit, Auiran).....	Methoxy ethyl mercury chloride.....	Bayer Products, Ltd., Agriculture Dept. Agrica House, Kingsway, London W. C. 2
Arathane WT-25.....	Ammonium salt of Dinitro Ortho Secondary Butyl phenol. Dinitrocapryl phenyl crotonate.....	Standard Agricultural Chem. Inc. Rohm and Haas Co.
Cadminate.....	Benzoic Acid.....	Baker's Chemicals
Cadmium Fungicide H 258.....	Organic cadmium compound.....	Malinckrodt Chemical Works
Cadmium Fungicide H 258-A.....	7.2 per cent cadmium.....	Merck and Co., Inc.
Ceresan.....	35-36 per cent cadmium.....	Merck and Co., Inc.
Ceresan M.....	Ethyl mercury chloride.....	E. I. Du Pont De Nemours & Co.
Compound 308.....	Ethyl mercury p-toluene sulfonamide.....	E. I. Du Pont De Nemours & Co.
Compound 629.....	Copper nitrodithio acetate.....	General Chemical Division, Allied Chemical and Dye Corp.
Compound 1189.....	Zinc nitrodithio acetate.....	General Chemical Division, Allied Chemical and Dye Corp.
CR-305.....	Chlorinated hydrocarbon.....	General Chemical Division, Allied Chemical and Dye Corp.
Crag Turf Fungicide No. 531.....	Copper 8-hydroxy quinolate (Technical). Bis (2 hydroxy-5 chlorophenyl) sulfide.....	California Spray-Chemical Corp. Rohm and Haas Co.
Crag Potato Fungicide 658.....	Calcium-zinc-copper-cadmium chromates.....	Carbide and Chemicals Corp. Carbide and Chemicals Corp.
Dow 9B Seed Protectant.....	Copper-zinc-chromates.....	Standard Agricultural Chem. Inc.
Experimental Fungicide 224.....	Dinitro Ortho Secondary Amyl phenol.....	Dow Chemical Company
Experimental Fungicide 640.....	Zinc trichlorophenate.....	Carbide and Carbon Chem. Corp.
F-800.....	7 Zn . 2HgO . 2CrO <sub>3</sub> . 7H <sub>2</sub> O.....	Carbide and Carbon Chem. Corp.
Ferbam (Nu-Leaf).....	ZnO . 4 CuO . CrO <sub>3</sub> . XH <sub>2</sub> O.....	Dow Chemical Company
Ferbam (Ferradow).....	Trichlorophenyl monochloro acetate 50%.....	California Spray-Chemical Corp.
Fungicide 1124.....	Ferrie dimethyl dithiocarbamate 70%.....	Dow Chemical Company
Good-rite V.L. 600.....	Ferrie dimethyl dithiocarbamate 76%.....	General Chemical Division, Allied Chemical and Dye Corp.
	Thiocynate (dinitrophenyl).....	B. F. Goodrich Chemical Co.
	Vinyl resin latex.....	

Name	Chemical Compound	Chemical Company
Hyamine 1622.....	Di-isobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride.....	Rohm and Haas Company
Hyamine 3258.....	.....	Rohm and Haas Company
KF 467.....	Organo-mercury compound.....	Dr. J. W. Heuberger, University of Delaware
Lignasan.....	Ethyl mercury chloride 6.25%.....	E. I. Du Pont De Nemours & Co.
Mercan B.....	Para-phenyl mercuric acetate.....	Pittsburgh Coke and Chem. Co.
Microgel.....	Tri-basic copper sulphate.....	Tennessee Corporation
Milner.....	8-quinolinol.....	Monsanto Chemical Company
Mycoban.....	Sodium propionate.....	E. I. Du Pont De Nemours & Co.
Nabam (Dithane D-14).....	Disodium ethylene bisdithiocarbamate hexahydrate 25%.....	Rohm and Haas Company
Ortho Mildew Spray.....	.....	California Spray-Chem. Corp.
Ortho Rix Spray.....	Calcium polysulfides 26% Polyethylene Glycol Mono-isooctyl phenylether 10% Para-nitrophenol.....	California Spray-Chem. Corp. Monsanto Chemical Company
Phygon.....	Phenyl mercury oleate.....	E. I. Du Pont De Nemours & Co.
Prentox (Seedox 50).....	Dichloronaphthoquinone.....	Naugatuck Chemical Division
Procop No. 110.....	2,4,5, Trichlorophenyl acetate 50%.....	R. J. Prentiss and Co.
Puratized Agricultural Spray.....	Copper naphthenate.....	The Harshaw Chemical Co.
Puratized 111-5.....	Phenyl mercury triethanol ammonium lactate.....	Gallowhur Chemical Corp.
Puratized GG.....	A mercury-copper quaternary ammonium complex.....	Gallowhur Chemical Corp.
Puraturf 177.....	Mercury-cadmium quaternary ammonium complex.....	Gallowhur Chemical Corp.
.....	Phenyl amino cadmium dilactate 20%.....	Mallinckrodt Chemical Works
.....	Pyridylmercuric acetate.....	Mallinckrodt Chemical Works
.....	Pyridylmercuric chloride.....	Mallinckrodt Chemical Works
.....	Pyridylmercuric stearate.....	Hercules Powder Co., Inc.
Scenesan.....	Rosin Amine D acetate.....	E. I. Du Pont De Nemours & Co.
Spergon.....	Hydroxymercichlorophenol 30%.....	Naugatuck Chemical Division
SR 406.....	Tetrachloro-para-benzoquinone 48%.....	California Spray-Chemical Co.
TAG Fungicide No. 331.....	.....	California Spray-Chemical Co.
.....	Phenyl mercuric acetate, 10% aqueous solution.....	Stecker Chemicals, Inc.
Thiosan.....	Tetramethyl Thiuramdisulfide 50%.....	E. I. Du Pont De Nemours & Co.
Vitamin K-5.....	2-methyl-4-amino-1-naphthol hydrochloride.....	Cutter Laboratories
Yellow Cuprocide.....	Cuprous oxide.....	Rohm and Haas Company
.....	Zinc Copper Chromate.....	California Spray-Chemical Corp.
Zineb (Dithane Z-78).....	Zinc ethylene bisdithiocarbamate 65%.....	Rohm and Haas Company
Ziram (Opalate, Zerlate).....	Zinc dimethyl dithiocarbamate.....	California Spray-Chemical Corp.

TABLE II

Percentage of germination and rotting of cuttings for the variety 32-8560, in which one-half of the plots were inoculated with *Ceratostomella paradoxa* and all plots irrigated immediately after planting (n=12).

Fungicide	Concentration <sup>a</sup>	Percentage of	
		Germination	Rotting
Pyridylmercuric acetate.....	1	75.7	0.8
" " .....	1/2	75.3	1.7
" " .....	1/4	68.3	5.0
" " .....	1/8	71.0	5.8
" " .....	1/16	68.0	7.5
" " .....	1/32	66.3	18.3
Pyridylmercuric chloride.....	1	76.0	2.5
" " .....	1/2	71.3	3.3
" " .....	1/4	74.0	4.2
" " .....	1/8	72.7	4.2
" " .....	1/16	66.7	11.7
" " .....	1/32	67.7	21.7
Ceresan.....	1%	73.0	14.2
Phenyl mercuric acetate.....	1 qt.	73.7	9.2
" " " .....	1 pt.	72.3	11.7
Para-phenyl mercuric acetate.....	2	71.3	5.8
" " " " .....	1	69.7	8.3
Seedox (Wettable).....	2	64.3	33.3
" " .....	1	70.0	35.8
Expt. Fung. 224.....	2	71.0	13.3
" " " .....	1	71.0	21.7
Puratized 111-5.....	2 qt.	71.0	5.0
" " .....	1 qt.	69.3	11.7
Hot-water treatment (HWT).....	50° C. 30 Min.	80.0	36.0
HWT+pyridylmercuric acetate.....	1/8	78.3	30.0
Check.....		62.0	47.5
		L.S.D.=6.7%	L.S.D.=10.4%

<sup>a</sup> All rates are given in ounces per gallon of water except for Ceresan used at 1% and for liquid fungicides which are listed as quarts and pints per 100 gallons of water.

TABLE III

Percentage of germination and rotting of non-inoculated cuttings and general vigor for the variety 38-2915, Oahu Sugar Co. (n=4).

Treatment	Concentration <sup>a</sup>	Percent		Vigor notes <sup>b</sup> (Av. 4 plots)
		Germination	Rotting	
Ceresan.....	1%	82.3	4.0	4
Pyridylmercuric acetate.....	1/8	79.5	3.5	4
" " .....	1/16	77.8	2.0	4
Pyridylmercuric chloride.....	1/8	76.8	2.8	4
" " .....	1/16	79.5	2.0	4
Phenyl mercuric acetate.....	1 qt.	82.0	3.0	4
Ceresan+HWT.....	1%	59.8	18.8	3
Pyridylmercuric acetate+HWT....	1/8	61.0	18.5	3+
Hot-water treatment (HWT).....	50° C. 30 Min.	38.9	28.3	2
HWT+pyridylmercuric acetate....	1/8	63.0	12.0	3
DDT.....	2.8%	58.0	14.8	3
Check.....		62.3	14.0	3
		L.S.D.=9.4%	L.S.D.=3.5%	

<sup>a</sup> Rates are given in ounces per gallon of water unless otherwise designated. Phenyl mercuric acetate was used at the rate of one quart per 100 gallons of water.

<sup>b</sup> Vigor is given on the basis of 1 to 5, where 1 represents very poor growth, 3 average, and 5 excellent growth of shoots.



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# Erosion as a Menace to the Sugar Industry of Hawaii

Roger P. Humbert<sup>1</sup>

Erosion losses have resulted in a continuous change in the surface of the earth since the beginning of time. Accelerated erosion has resulted from man's activities in the field of agriculture. In recent years, man's attention has been focused on the problem of erosion principally by alarmists who continue to paint pictures of tragedies.

The problem of erosion control in Hawaii is of such a serious nature as to cause universal concern. Sickness has struck in our home, and it is time the symptoms were diagnosed and corrective measures taken. Unprotected fields are being churned into countless rivers of mud which are carrying fertile topsoil to the sea. An inspection of these fields after heavy rains reveals thousands of tiny columns of earth sticking up above the soil surface. Each little pillar has a roof, either a pebble or a piece of vegetation (Figure 1).

This is the result of one type of erosion—"splash erosion." Careful observations by Ellison (1) have served to emphasize the importance of "splash erosion." Raindrops falling on unprotected soil tear particles loose from clods and other structural aggregates, and carry them into the air in suspension. (See cover picture and Figure 2). These particles often move through the air for distances of several feet and fall into the shallow rivers of moving water. According to measurements of splash erosion made by Ellison on a 10 per cent slope and in the absence of wind, three times as much soil moved downslope as moved upslope.

Nichols and Gray (2) calculated that rain falls at a speed of about 20 miles per hour. A two-inch rain falling at this rate contains enough energy to lift a seven-inch layer of soil three feet. Fortunately, this energy is spread out over considerable time and comes as millions of little explosions (Figure 2).

During a rainstorm, soil and water on an unprotected field are kept in constant turbulence. The pores of the soil surface become plugged by individual particles which orient themselves on settling, thus reducing the rate of entry of water into the soil. This process of surface sealing develops very quickly in certain types of soil, and by destroying the water-absorbing potential of the soil, sets the stage for accelerated erosion. The churning water, unable to find entry into the soil, fills the pockets on the field and then begins to run off. The moving water, with the soil it holds in suspension, scours more soil loose as it moves downslope. As water gathers in deeper channels, it moves faster and faster. The faster it moves, the more soil it can hold in suspension.

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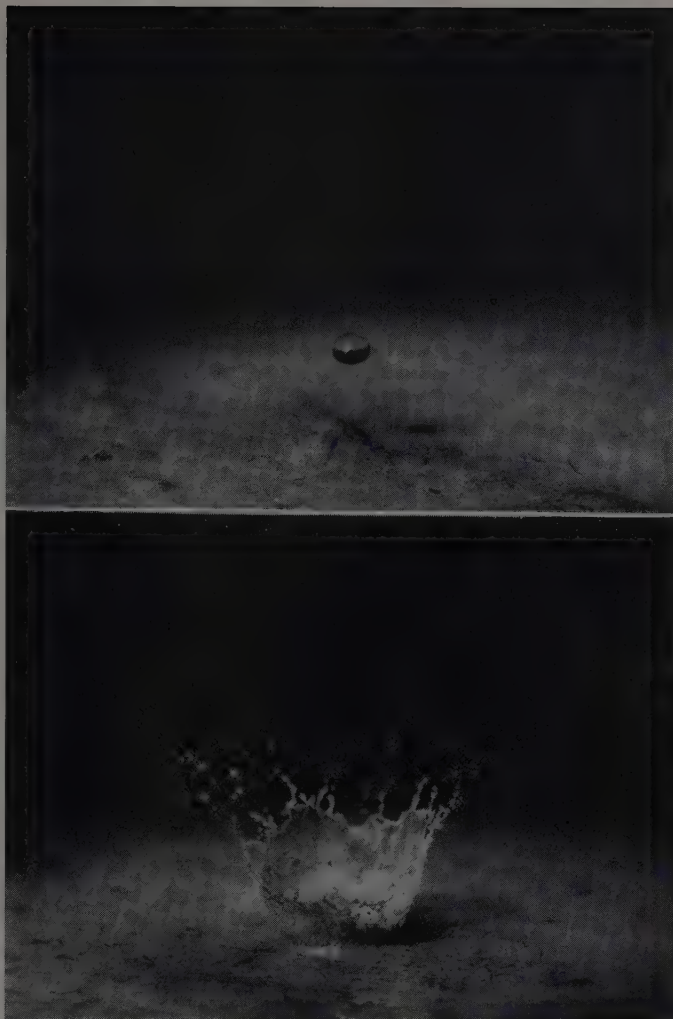


Figure 2. Top: Raindrop about to strike the surface of wet soil. Bottom: Miniature explosion caused by raindrop. (Courtesy Naval Research Laboratory)

Figure 3 illustrates the damage that is commonly observed in all the islands during periods of heavy rainfall. In a field of young cane at Wailuku, fertile soil has been removed to the depth reached by the subsoil points of the plow during preparation of the soil. In such tillage operations, care should be taken to operate across possible future channels of moving water.

Figure 4 shows erosion in a field of young cane at Oahu Sugar Company. The water had accumulated in the hollow and eventually had broken through the cane lines. The rapidly running water quickly turned into a river of red mud. In a nearby



Figure 3. Erosion in sugar cane field at Wailuku Sugar Company, December 1950.

field of older cane, under comparable conditions of soil and slope, runoff was greatly reduced. In this second case, water in the runoff at the bottom of the field was moving at a much slower rate and was practically clear. The canopy of cane had destroyed the energy of the falling raindrops, which, upon reaching the ground, infiltrated into the soil.

On the irrigated plantations, it becomes necessary to send repair crews to the fields following each heavy rain. The breaches in the lines must be filled with soil before the advent of either another rain or the next round of irrigation. In the un-irrigated plantations, although the damages do not go unobserved, the frequency of heavy rains often results in an inadequate program of control.

Gullies, if permitted to grow unchecked, will eventually develop into gulches like those which now mar the landscape of the Hilo and Hamakua coast region (Figure 5). Erosion has been directly responsible for the removal from sugar production of sizable areas, and is taking its toll in fields now under production by expanding the areas of unproductive knolls. Figure 6 shows a corn field in Michigan—yet it could represent equally well hundreds and hundreds of spots in the fields of many of the plantations.

The importance of erosion in the knoll problem of the Hilo and Hamakua coasts cannot be overemphasized! More and more infertile subsoil is being exposed as erosion takes its toll and as the number and extent of the knolls grow. The surface soils are too thin in many places to support a good crop of cane. Recent studies have shown the roots to be confined largely to the surface soil, as they refuse to venture into the claypans and infertile subsoils that lie below. Every effort must be made to preserve this thin blanket of fertile surface soil which is the lifeline of the Hilo coast.

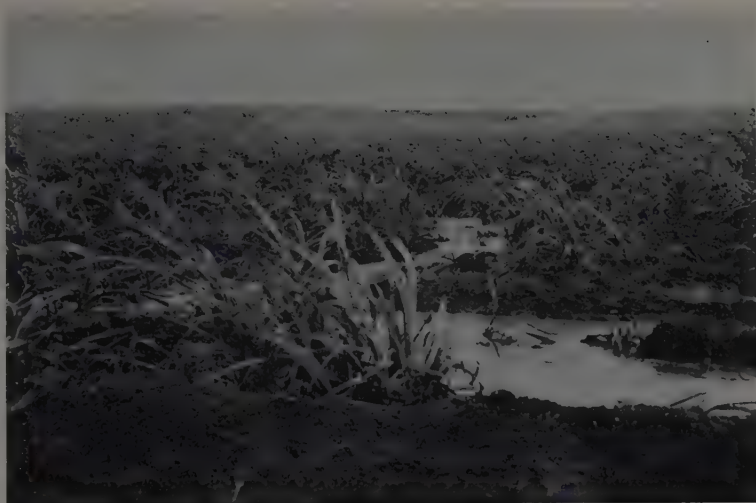


Figure 4. Erosion in field of young cane at Oahu Sugar Company, March 1951.

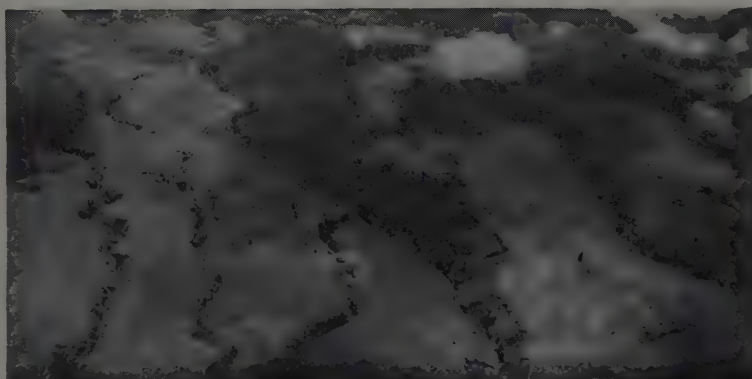


Figure 5. Aerial photographs of eroded sugar cane fields on the Hilo and Hamakua coasts of Hawaii.



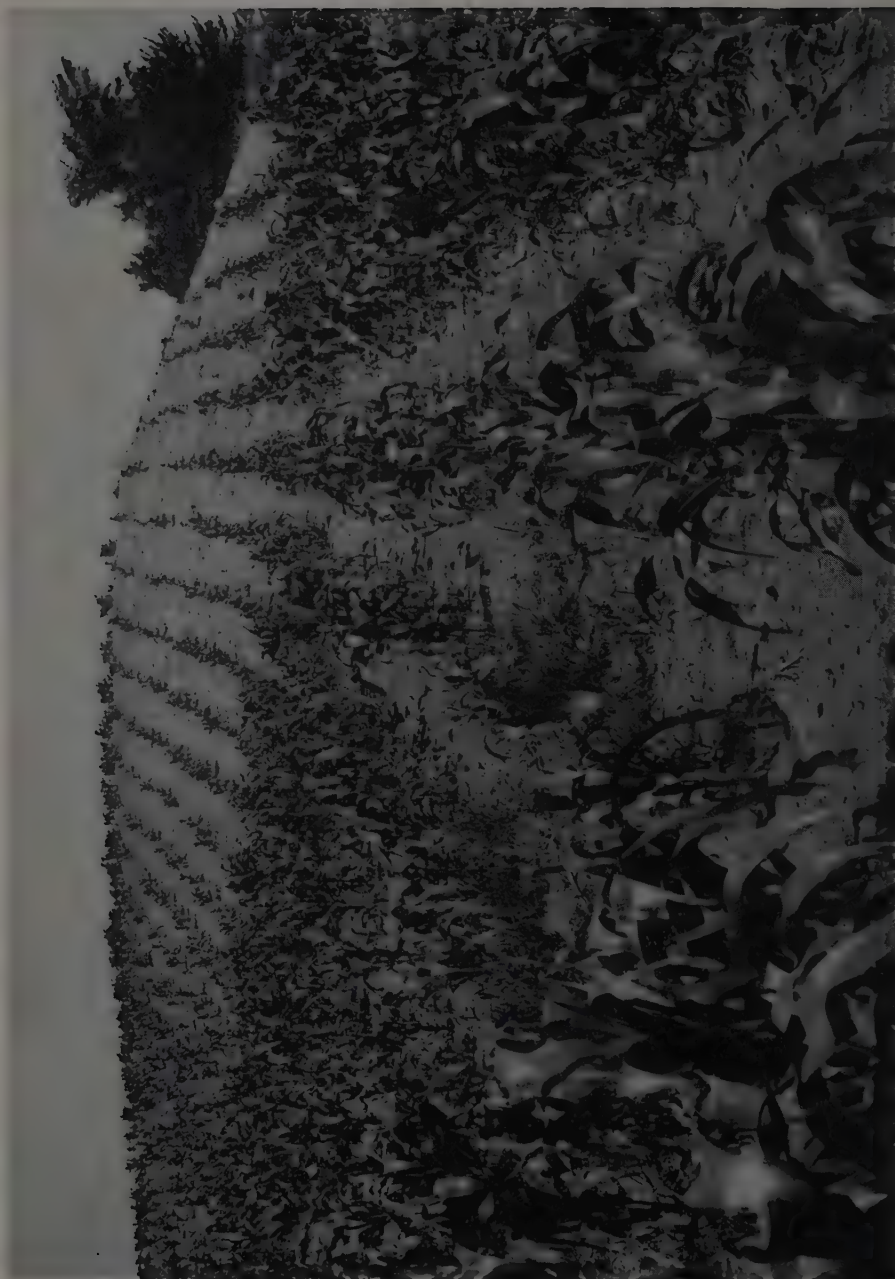


Figure 6. Corn field in Michigan. Knoll problems in the corn belt are similar to those of the sugar industry in Hawaii.  
(Courtesy Soil Conservation Service)



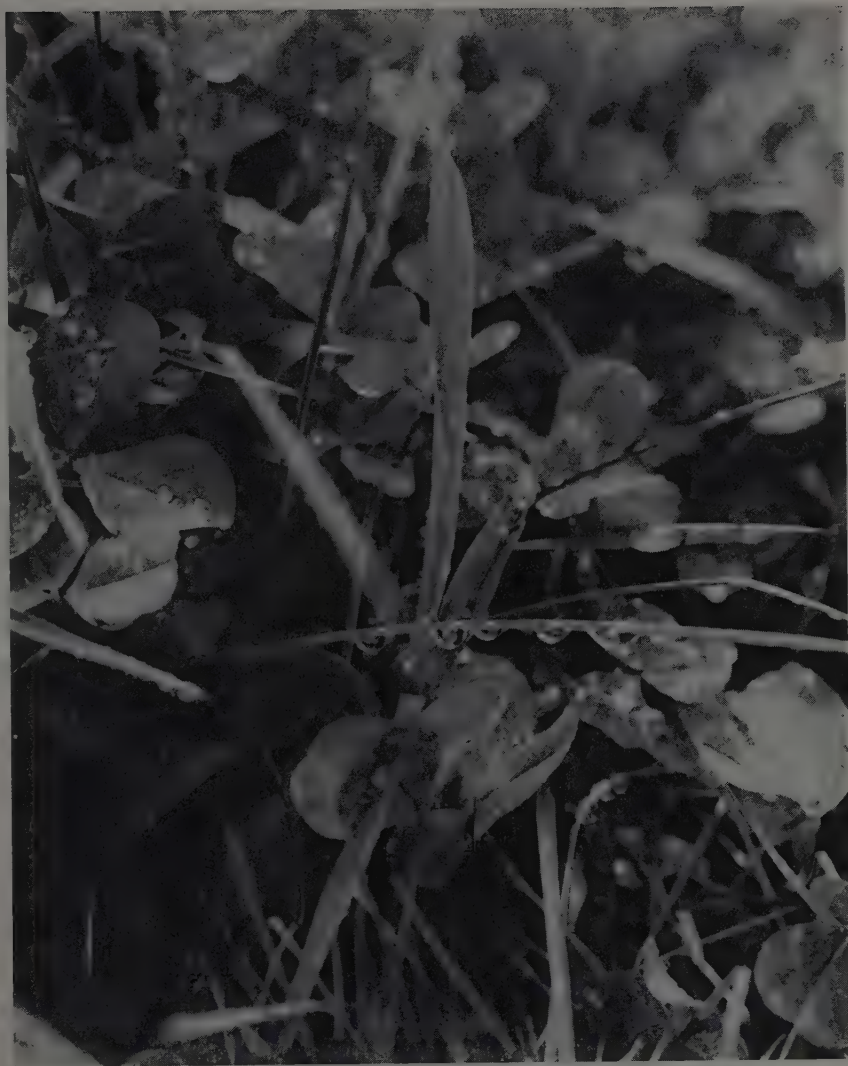


Figure 7. Cover of vegetation is effective in destroying the energy of falling rain.  
(Courtesy Soil Conservation Service)

## THE CONTROL OF EROSION

Erosion control should start with the control of the raindrop. Cover is the key to de-energizing the raindrop effectively. Figure 7 illustrates the effectiveness of a blanket of vegetation in absorbing the shock of falling rain. Sugar cane itself affords similar protection, once its canopy is complete.

Vegetation need not be alive to be effective. Surface mulches of organic materials are excellent protection against the beating action of rain. The effectiveness of a thin trash mulch on the surface can be seen in the two lower containers in Figure 8. The mulch cushions and eliminates most of the drop impact, thus preventing the dispersal of clods and aggregates, and in so doing, helping to preserve the infiltration capacity of the soil.

Factors which determine the extent of erosion are:

1. Intensity and duration of rainfall.
2. Type of soil.
3. Length and degree of slope.
4. Amount and character of cover.

Until man learns to disperse clouds and regulate the distribution of rainfall, there appears to be little hope of controlling rainfall. Efforts should therefore be directed to intelligent management of the other three factors.

The types of soils and their physical properties define the entry and movement of water through their profiles. Over large areas of the high-rainfall belts of Hawaii, the soils were originally richer in organic matter than they are at present. Continuous use of these soils in the production of sugar cane has resulted in substantial losses of organic matter.

Where do these losses fit into the picture of sugar cane production? The facts show that the productive capacity of sugar cane soils is deteriorating at a rate which offsets gains made both by improved cane varieties and by better cultural practices. This soil deterioration can unquestionably be blamed to the loss of organic matter and the resulting degradation of the soil's physical properties. Every effort must be made through intelligent soil management to keep the fertile topsoil in place and to prolong its productive life.

Because of the limited amount of arable land and the high value of the crops produced, areas have been thrown into production which should never have been exposed to the forces of erosion. These areas are rough and sloping in character, without much natural protection once their surfaces are exposed. Erosion could be curtailed in many such areas if the necessary cover were furnished by means of strip cropping with canes of different ages. The limitations of such a system of management would undoubtedly define the size of the sections into which a field unit could be economically handled.

When cane reaches the "closing in" stage, it furnishes a protection which reduces erosion losses to a minimum. The condition persists until the cane is harvested. Following harvest and subsequent tillage operations, the soils are again in a vulnerable condition. It is at this stage that the raindrop becomes destructive. Since the raindrop loses much of its power if its fall is cushioned, every effort should be expended to use a blanket of cane trash as a shock absorber.

If, through programs of good soil management, the raindrop can be controlled and made to seep into the soil, erosion as a menace to the sugar industry of Hawaii will disappear.

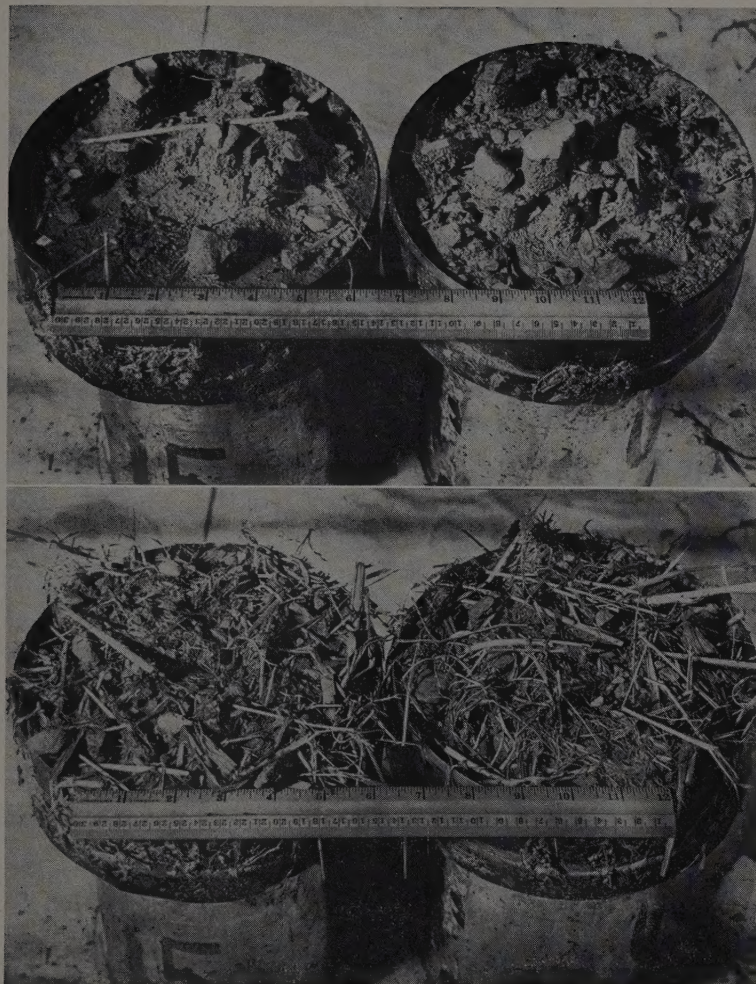


Figure 8. Top: Exposed soil after period of heavy rain. Bottom: Soil protected by thin trash mulch after same period of heavy rain.

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- <sup>2</sup>Nichols, M. L. and Gray, R. B. Some important farm machinery and soils conservation relationships. Agri. Eng. Vol. 22: pp. 341-343. 1941.



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